THE CHEMISTRY OF THE BILE ACIDS AND RELATED SUBSTANCES

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I. INTRODUCTION

The knowledge of the constitution of the bile acids and the sterols has recently (1932) been revolutionized. The hypothetical structural formulas developed step by step through the continuous experimental efforts of Wieland, Windaus, and others during the last twenty years had to be fundamentally revised on the basis of new evidence. Adumbrated by certain findings of Diels, the new conception grew out of x-ray spectroscopy (13). Rosenheim and King "supplied the spark which has led to such a notable bonfire of unseemly molecular structures." (Robinson, in reference 51.) In the words of the English investigators, their contribution "would have been impossible but for the fundamental experimental work which forms the basis of present-day knowledge and for which we are indebted to the unremitting labors of Mauthner, Windaus, Diels, Wieland, Borsche, Schenck, and their collaborators" (156). It may be added that during the past two years the German and the British groups have assiduously translated most of the older evidence into terms consistent with the new conception. Thus, most discrepancies, unaccounted for by the old formulas, have vanished and a rich crop of additional facts harmonizes with the revised interpretation of the older.

The physiology of the bile acids and sterols has advanced steadily abreast with the progress of the medical sciences, especially experimental surgery and pharmacology, but one can hardly fail to observe the wide gap between these biological investigations and the chemical research. Yet the greater lucidity, recently attained in the chemistry of the bile acids, should dispel the reluctance of physiological chemists to link the remarkable chemical characteristics of these substances with their equally noteworthy place in the physiology and pathology of the animal organism. These bridges will be built along three lines of thought, two of which, while comparatively young, are sufficiently advanced to warrant their application. First, colloidal chemistry, or more properly, capillary chemistry, the importance of which for the extremely surface-active bile acids requires no comment. Second, the chemistry of molecular or coördination compounds, inaugurated by Werner, and applied to organic substances particularly by Pfeiffer. It was this concept which inspired Wieland to establish the "choleic acid principle" (200). The third idea which rather inheres in this special branch of organic chemistry, is that of the genetic relation between bile acids and sterols, presaged perhaps for the first time by Lachinov (111), and corroborated through a steadily converging series of investigations, culminating in the interconversion of bile acids and sterols, and stimulated by the discovery of such physiologically active substances of related structure as vitamin D, the sex hormones, and certain carcinogenic substances.

At this juncture, a review must reveal a vista from the point that has

been reached, giving proofs for the new ideas rather than explanations for past errors. This does not preclude the value and the necessity of consulting the older sources for the student who wishes to acquaint himself with the innumerable experimental facts on record, but is eager to interpret them in the light of modern knowledge.[1]

II. OCCURRENCE OF BILE ACIDS IN NATURE

Bile acids are the preponderant constituent of bile, the secretion of the liver in vertebrate animals. They occur in conjugated form, i.e., in a peptide-like linkage with the amino acids glycine and taurine. Toad-poison, bufotoxin, contains arginine and suberic acid in conjugation with bufotalin which is a bile acid derivative. The lowest order of fish excretes in its bile the "biloid" scymnol as a sulfuric acid ester. The significance of conjugation, which imparts greater water-solubility, is beyond the scope of this review. It may be said, however, that the liver uses conjugation also for purposes of elimination, e.g., in the case of benzoic acid. Synthetic conjugation with glycine and taurine was verified for cholic acid by Bondi and Müller (18) using Curtius' method (cholic acid ethyl ester → cholyl hydrazide → cholyl azide → cholyl glycine or cholyl taurine), and for deoxycholic acid by Wieland (202).

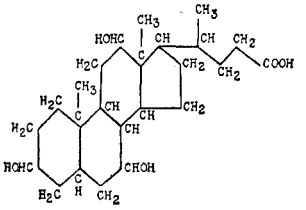
The ratio of taurine to glycine derivatives in bile varies from species to species; hog bile contains practically no taurine, while the bile of dogs, fish, snakes, etc., is devoid of glycine. Human bile and ox bile contain approximately equal amounts of tauro- and glyco-bile acids.

The cleavage of the peptide bond is presumably effected in nature by an enzyme (cf. 79, 131). In the laboratory, it is accomplished by alkaline hydrolysis. The resulting nitrogen-free unconjugated, or simply "free," bile acid consists of a mixture of acids in proportions which vary with species and with local and seasonal factors. At least half a dozen natural free bile acids have been isolated. The most frequent are cholic acid and deoxycholic acid; hyodeoxycholic acid prevails in hog bile, and chenodeoxycholic acid in goose bile.

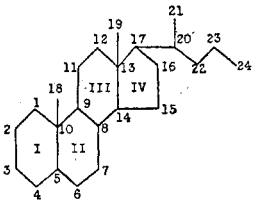
Constitution

Cholic acid (I) is a saturated trihydroxymonocarboxylic acid derived from the hydrocarbon cholane, $C_{24}H_{42}$ (II), which is eight hydrogen atoms poorer than the corresponding paraffin. Therefore, it must have four rings. Its formula is as follows (155, 229, 267).

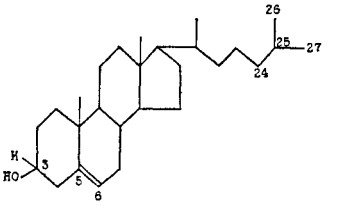
[1] A simplified nomenclature in this field was proffered in references 158 and 161, and has been followed in the present review. Summaries of recent chemical developments are given in references 161, 94, and 267; discussions of the biochemical aspects may be found in references 178 and 145.



I
Cholic acid,
$C_{24}H_{40}O_5$



II
Cholane,
$C_{24}H_{42}$



III
Cholesterol,
$C_{27}H_{46}O$

The numbering of the carbon atoms and of the rings is conventional; the position of the two methyl groups is not definitely proven. Cholesterol (III), the chief representative of animal sterols, is a secondary monounsaturated tetracyclic alcohol, $C_{27}H_{45}OH$, whose formula will be discussed simultaneously with that of the bile acids (155; cf. 185).

III. METHODS OF STRUCTURAL INVESTIGATION

Before surveying the arsenal of reactions which serve as tools in the structural investigations, one should mention that the development of organic microanalysis was a direct outcome of Pregl's researches on the bile acids (120). It was essential for the achievements of bile acid chemistry, where a long sequence of reactions, despite fair yields, reduces kilograms of starting material to mere grams of degradation products holding promise of unequivocal information. On the other hand, physical methods, such as measurements of unimolecular surface films (1, 2), x-ray spectroscopy (13), and ultra-violet absorption spectra (133, 157), have guided the structural research at important crossroads.

A. Oxidation; dehydrogenation

The foremost place among chemical reagents employed in disentangling the carbon skeleton of bile acids and sterols is occupied by oxidizing agents.

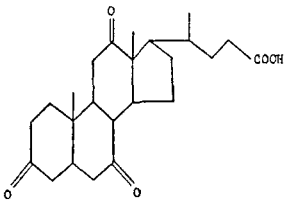
Chromic acid, CrO_3, in sulfuric or acetic acid solutions of high concentrations, is the most specific oxidant for the conversion of secondary alcoholic hydroxyl groups into keto groups, when used at low temperatures.

The classical example for its application to the present subject remains the dehydrogenation of cholic acid by Hammarsten (87) to a triketo acid known as dehydrocholic acid (IV). The reaction products of this method are regularly referred to as dehydro acids. Their functional nitrogenous derivatives, such as oximes, semicarbazones, and hydrazones, are extremely useful for the characterization of the parent substances. As will be seen later, these keto acids lend themselves better to reduction than the hydroxy acids and are indispensable intermediates in the replacement of secondary alcohol groups by methylene groups. Their isolation is desirable, although not imperative, when the destruction of a ring by stronger reagents, such as chromium trioxide at higher temperatures, nitric acid, or bromine, is contemplated. These three reagents break up the grouping $—CH_2—CO—$ into $—CO_2H\,|\,HO_2C—$, sometimes via the diketone —CO—CO— which has been isolated in rare instances (e.g., the bilisoidanic acid of Schenck (167)).

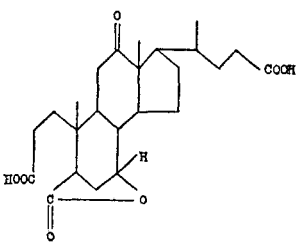
The mechanism of oxidation by bromine, which was first attempted by Landsteiner (117), has been studied extensively, and many mono- and dibromo derivatives of the —CHBr—CO— and —CHBr—CO—CHBr— type have been isolated, especially by Wieland and Dane (44, 226). These α-bromoketo substances undergo numerous reactions, such as replacement of bromine by hydroxyl, and, depending on the presence of neighboring methylene groups, loss of hydrogen bromide yielding —CH=CH—CO, and enolization yielding enediols.

$$—CHOH—CO— \rightarrow —COH=COH—$$

The action of bromine on hydroxyl groups is peculiarly specific; in the case of cholic acid, bromine will disrupt ring I between carbon atoms C_3 and C_4 as outlined above, it will oxidize the hydroxyl group on C_{12} to a carbonyl, but it will leave the hydroxyl on C_7 untouched so that the intervening lactonization of this hydroxyl with the carboxyl C_4 leads mainly to the formation of biliobanic acid (V) (144, 212).

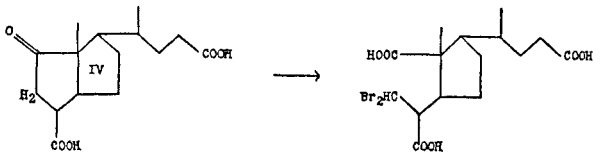


IV
Dehydrocholic acid,
$C_{24}H_{34}O_5$



V
Biliobanic acid,
$C_{24}H_{36}O_7$

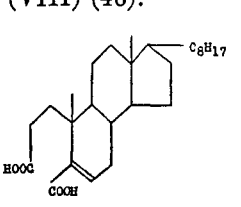
The action of bromine on methyl ketones, resulting in the splitting-off of bromoform, finds its counterpart in the effect of bromine on a keto derivative (VI), which is converted into an ω-dibromocarboxylic acid (VII) (224).



VI
Ketodicarboxylic acid,
$C_{15}H_{22}O_5$

VII
Dibromotricarboxylic acid,
$C_{15}H_{22}O_6Br$

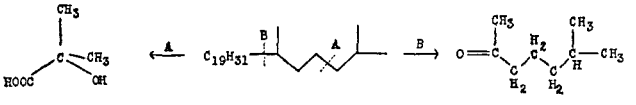
The use of potassium hypobromite on cholesterol leads to a clean scission of ring I between C_3 and C_4 (VIII) (46).



VIII
Diels' acid,
$C_{27}H_{44}O_4$

The most vigorous, but least lucid oxidations are effected with nitric acid of specific gravity 1.40–1.42 or fuming nitric acid of specific gravity 1.52. They lead to the opening of two or all of the three rings (I, II, and III) and, while the yield of a specific desired reaction product is not so advantageous as a rule, nitric acid still deserves its place in this field. In some instances, intermediates containing a nitro group have been isolated; for instance, nitrocholestenone, where the nitro group is attached to an "aromatic" carbon atom.

Nitric acid, and subsequently chromium trioxide, were applied by Windaus (246, 247, 249) in the destructive oxidation of the side chain of cholesterol, leading to the isolation of acetone, α-hydroxyisobutyric acid (IX), and methyl isohexyl ketone (X) (248).

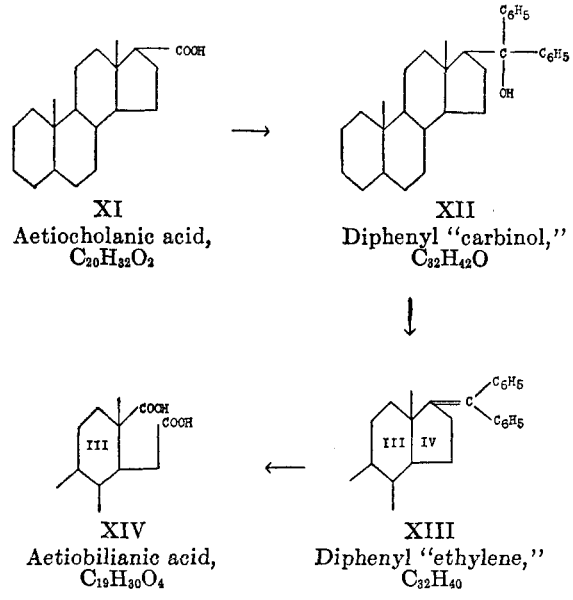


IX
α-Hydroxyisobutyric acid,
$C_4H_8O_3$

III
Cholesterol

X
Methyl isohexyl ketone
$C_8H_{16}O$

Chromium trioxide was also the oxidizing agent used by Wieland, Schlichting, and Jacobi (221) in the stepwise Grignard degradation of the side chain in cholanic acid, and it was with this reagent that the first and so far only inroad was made into ring IV after the side chain was replaced in the last step by $\rangle C{=}C(C_6H_5)_2$ (XI to XIII), leading to the formation of two new carboxyl groups in aetiobilianic acid (XIV). For the formation of *n*-butane-1,3,3′-tricarboxylic acid from Wieland's diketodicarboxylic acid (CXXXII from CII), see p. 344.



XI
Aetiocholanic acid,
$C_{20}H_{32}O_2$

XII
Diphenyl "carbinol,"
$C_{32}H_{42}O$

XIV
Aetiobilianic acid,
$C_{19}H_{30}O_4$

XIII
Diphenyl "ethylene,"
$C_{32}H_{40}$

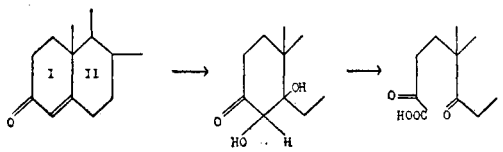
Potassium permanganate differs from the previous oxidizing agents in its action on keto groups. It will sever a carbonyl group from a tertiary rather than from a secondary carbon neighbor. In the model substance α-methylcyclopentanone, it produces acetylbutyric acid (δ-ketocaproic acid) rather than methylpimelic acid (196). This type of ring cleavage, while especially frequent with permanganate (Wieland's diketodicarboxylic acid (CII), $C_{23}H_{34}O_6$, from pyrodeoxybilianic acid (CI)), has been encountered occasionally in oxidations with nitric acid.

Neutral permanganate, for instance, in acetone solution also leads to the formation of a keto acid in cases of α,β-unsaturated ketones like coprostenone

$$-CO-CH{=}C\rangle \rightarrow -CO-COOH\,\vdots\,OC\rangle \rightarrow -CO_2H\,\vdots\,CO_2\,\vdots\,OC\rangle$$

through a series of hypothetical intermediates (XV to XVIII), which at

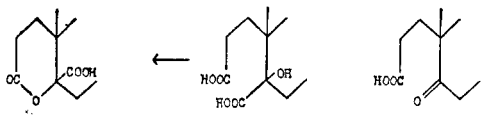
the same time may serve to explain the formation of the lactone acid, $C_{27}H_{44}O_6$, in the example given below (XIX to XX) (185, 245).



XV
Coprostenone, $C_{27}H_{44}O$

(XVI)
(Dihydroxycholestanone, $C_{27}H_{46}O_3$)

(XVII)
(Diketo acid, $C_{27}H_{44}O_4$)



XX
Lactone acid, $C_{27}H_{44}O_6$

(XIX)
(Hydroxydicarboxylic acid, $C_{27}H_{46}O_7$)

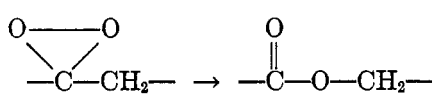
XVIII
Keto acid, $C_{26}H_{44}O_3$

Cyclic α-hydroxy acids, on treatment with permanganate in sulfuric acid, will yield cyclic ketones through the loss of the elements of formic acid (197); see p. 330, formula for cilianic acid (LXIII).

While potassium permanganate is the traditional oxidant for aliphatic double bonds (Baeyer's reagent), the cleanest method for the introduction of two vicinal hydroxyl groups uses hydrogen peroxide, for instance, in the preparation of cholestantriol (143, 253); benzoyl peroxide may also be used (198). A peculiar reaction is the oxidation of cyclic ketones, $-CO-CH_2-$, by ammonium persulfate (9), yielding a primary hydroxy acid

$$-COOH \vdots HOH_2C-$$

presumably (244) via

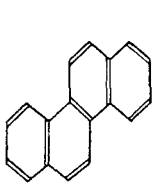


The appearance of acetone in peroxide oxidations is not confined to isopropyl derivatives; acids with secondary methyl groups, such as α-methylglutaric acid, also form acetone (224).

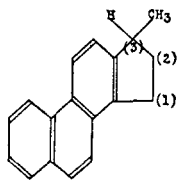
Ozone has been applied to the double bond of cholesterol by Dorée and Gardner (54, 56) and Dorée and Orange (57; cf. 90). Butenandt used ozone in his studies on pregnandiol (30). Levorotatory isopropylmethyl-

acetaldehyde has been obtained from ergosterol, and isopropylethylacetaldehyde from stigmasterol by the action of ozone upon the double bond between C_{22} and C_{23} in these sterols (150, 82, 83).

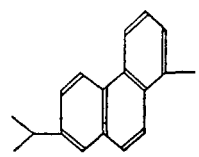
Dehydrogenation proper, resulting in the formation of double bonds, has been studied especially by Diels (47). When dehydrogenation of saturated hydrocarbons and their halogen derivatives with palladized charcoal catalysts proved unsatisfactory, Diels resorted to sulfur (cf. 190). As this element tends to insert itself into cyclic structures at the elevated temperatures of the reaction, he replaced it by selenium. Dehydrogenation of cholesterol by selenium yielded two hydrocarbons, 3-methylcyclopentenophenanthrene (XXI), $C_{18}H_{16}$, and $C_{25}H_{22}$ (or H_{24}), while palladized charcoal on cholesterol or selenium on cholic acid led to a hydrocarbon, $C_{18}H_{12}$, identified by Diels (48, 49, 50) as chrysene (XXII). These aromatic and semiaromatic hydrocarbons contain the tetracyclic skeleton of cholesterol. The methyl groups attached to tertiary carbon atoms were eliminated as methane, or shifted from C_{13} to C_{17}, in the course of dehydrogenation (aromatization), and the side chain was either dropped, giving rise to paraffins such as propane, hexane, and isoöctane, or a new ring was formed from it. These aromatizations of polycyclic compounds are not always complete, depending on the catalyst employed. It has been pointed out by Rosenheim and King (156), "that dehydrogenation with selenium leads to the fully aromatic hydrocarbon, $C_{18}H_{12}$, i.e., chrysene, when applied to cholic acid with its three alcoholic hydroxyl groups, symmetrically disposed in three rings, whilst the same process stops at the partially saturated hydrocarbon, $C_{18}H_{16}$, in the case of the sterols (cholesterol and ergosterol), where the point of attack is limited to a single hydroxyl group in one of the outside rings." Additional evidence along these lines may be expected from the studies on abietic acid, β-pimaric acid, the hydrocarbon retene (XXIII), and other partly hydrogenated phenanthrane derivatives (91).



XXII
Chrysene, $C_{18}H_{12}$



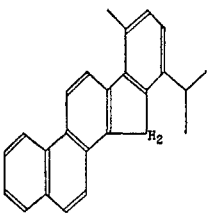
XXI
3-Methylcyclopenteno-phenanthrene, $C_{18}H_{16}$



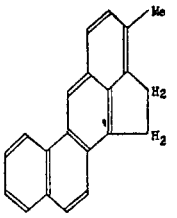
XXIII
Retene, $C_{18}H_{18}$

The belief that the aromatic products obtained by these methods are not due to a complicated pyrosynthesis, but represent the true carbon skeleton of cholesterol and of the bile acids, has been corroborated (e.g., see reference

147). Ruzicka, Goldberg, and Thomann (164) have cast some doubt on the identity of $C_{18}H_{12}$ from cholesterol with chrysene, but spectroscopic evidence (50) supports it. The identity of $C_{18}H_{16}$ with 3-methylcyclopentenophenanthrene has been confirmed by comparison of the substance with synthetic cyclopentenophenanthrene (40), 1-methyl- and 2-methylcyclopentenophenanthrene (108, 164), and 3-methylcyclopentenophenanthrene (10, 89, 14). The oxidation of $C_{25}H_{22}$ with chromium trioxide yields the monoketone $C_{25}H_{20}O$ (XXIV). Aromatic six-membered rings give rise to quinones under these conditions, whilst five-membered rings of the fluorene type form monoketones. These facts, suggesting the original presence of a five-membered ring, are in agreement with other experimental evidence concerning ring IV of sterols and bile acids (157). Another pentacyclic derivative, dehydronorcholene (LX), whose formation from bile acids will be derived on p. 329, yields on dehydrogenation a hydrocarbon, $C_{21}H_{16}$, methylcholanthrene (236, 39), which was found by the British workers to be a most active carcinogenic substance (cf. p. 354 and formula XXIV A).



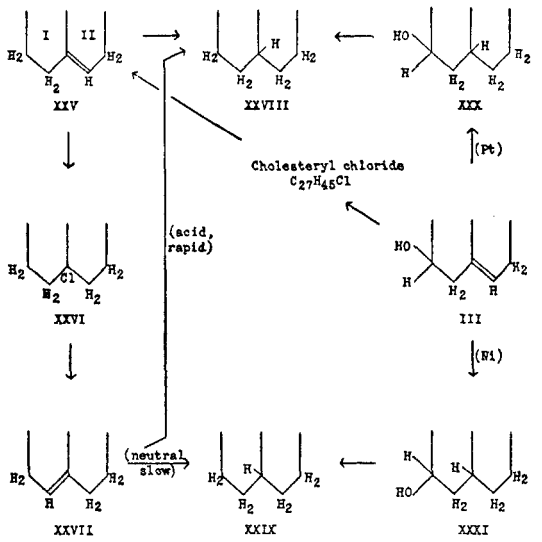
XXIV
Hydrocarbon $C_{25}H_{22}$ according to Rosenheim and King (157)



XXIV A
Methylcholanthrene, $C_{21}H_{16}$

B. Hydrogenation; reduction

Among the various methods of hydrogenation, one has to enumerate catalytic reduction by hydrogen in the presence of palladium, platinum, or nickel at normal or elevated temperature and pressure. These catalysts have been used widely, especially in the hydrogenation of double bonds. Several members of the sterol series, such as cholesterol itself, are endowed with double bonds. If one of the double bonded carbon atoms is tertiary, i.e., if it carries no hydrogen atom, then the addition of H—H will give rise to a new center of asymmetry and two dihydro compounds may theoretically be formed (cf. 173). In reality, this will occur with some hydrogenating agents, whilst others will lead to the preferential formation of one isomer. The interrelations between cholestene (XXV), coprostene (pseudocholestene) (XXVII), cholestane (XXVIII), and coprostane (pseudocholestane) (XXIX) may serve as an example.



III. Cholesterol, $C_{27}H_{46}O$
XXV. Cholestene, $C_{27}H_{46}$
XXVI. Cholestene hydrochloride, $C_{27}H_{47}Cl$
XXVII. Coprostene, $C_{27}H_{46}$
XXVIII. Cholestane, $C_{27}H_{48}$
XXIX. Coprostane, $C_{27}H_{48}$
XXX. Cholestanol (dihydrocholesterol or β-cholestanol), $C_{27}H_{48}O$
XXXI. Epicoprostanol (δ-cholestanol), $C_{27}H_{48}O$

The scheme illustrates at the same time the shift of double bonds through intermediary addition and subtraction of HCl, first observed by Mauthner (130). Another example for this reaction is given by Rosenheim and King (156), who, in unpublished experiments with Starling, converted apocholic acid by hydrochloric acid into the structural isomer dihydroxycholenic acid.

The hydrogenation of cholesterol itself in the presence of platinum yields cholestanol (dihydrocholesterol), according to Willstätter and Meyer (243), while the reduction with nickel as catalyst results in a mixture (γ-cholestanol) of cholestanol (β-cholestanol) and epicoprostanol ("δ-cholestanol").

Other unsaturated compounds are formed especially from bile acids by the loss of H—OH from the grouping $R_1R_2CH—COHR_2R_3$, resulting in $R_1R_2C{=}CR_3R_4$. If neither R_1 nor R_2 represents a hydrogen atom, then atom *C* must have been a center of asymmetry in the hydroxy compound. This asymmetry disappears on dehydration and is restored through the subsequent hydrogenation of the ethenoid linkage. Again, hydrogenation potentially yields two stereomers. In the case of multiple unsaturation, as in ergosterol among natural compounds or in cholatrienic acid among synthetic derivatives, at least eight stereomers may form according to the

theory. It seems, however, that the hydrogenation of such entwined polycyclic structures is governed by certain laws of mutual induction and leads to the preferred formation of a very limited number of products, frequently just of a single isomer.

The course of the hydrogenation of keto groups, particularly in the aforementioned dehydro acids, depends likewise on the method used. Electrolytic reduction of dehydrocholic acid, or action of sodium amalgam or aluminum amalgam in ether–benzene, leads to 3-hydroxy-7,12-diketocholanic acid (reductodehydrocholic acid), while Willstätter's platinum catalyst yields 3,7-dihydroxy-12-ketocholanic acid plus 3,7,12-trihydroxycholanic acid (cholic acid), which is the sole reaction product when platinum at 80–90°C. and hydrogen at 3 atmospheres pressure is used (Skita's method). One of the most successful hydrogenation methods is that developed by Clemmensen in the laboratories of Parke, Davis and Co. (35). Clemmensen used zinc amalgam in the presence of hydrogen chloride in glacial acetic acid for the reduction of keto groups in aliphatic, hydroaromatic, aromatic, and polycyclic compounds. For example, zinc amalgam will reduce dehydrocholic acid to cholanic acid. While, according to Clemmensen's experience, aromatic hydroxyl groups are left intact (aromatic oxyketones → phenols), hydroaromatic secondary alcohol groups are reduced; for instance, in the case of reductodehydrocholic acid the hydroxyl group, as well as both keto groups, is reduced, yielding cholanic acid.

The complete reduction of carbonyl, with secondary alcohol groups remaining unchanged, can be accomplished by the procedure of Kishner (106) and of Wolff (268). The hydrazone or preferably the semicarbazone is prepared; absolutely anhydrous sodium ethylate will then replace the $=N—NH_2$ or $=N—NH—CO—NH_2$ by H_2, without any reaction on the hydroxyl group. Double bonds are left intact both by Kishner-Wolff's and by Clemmensen's method. The degree of resistance of keto groups against hydrogenation varies with the position of the carbonyl. Those in ring I, especially on C_3, are the most reactive; those in ring III, on C_{12}, the least reactive. This factor can be utilized in various ways for a great variety of preparative purposes in combination with partial dehydrogenation and dehydration. Parallel differences of reactivity will also be found in the tendency of the various carbonyl groups to form oximes and other functional derivatives.

Sodium metal in alcoholic solutions and sodium ethylate reduce carbonyl groups to carbinol. Their occasional hydrogenating action on double bonds in unsaturated alcohols like cholesterol seems to be due to intervening rearrangements into the respective saturated ketone (e.g., cholesterol → cholestanone → γ-cholestanol).

The replacement of halogen by hydrogen offers no peculiarities in the case of halogenated bile acid derivatives.

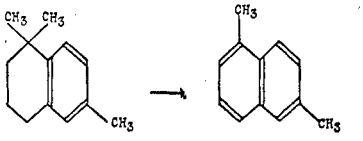
C. Dehydration

A considerable number of unsaturated acids have been prepared from the various natural hydroxy acids of bile by distillation in a high vacuum. The removal of the constituents of water from —CHH—CHOH— may take place in several stages, depending on the number of secondary alcohol groups in the molecule. Thus, lithocholic acid (3-hydroxycholanic acid) gives $\Delta^{2,3}$- or $\Delta^{3,4}$-cholenic acid, the several dihydroxycholanic acids, (3,7- or chenodeoxycholic acid, 3,6- or hyodeoxycholic acid, 3,12- or common deoxycholic acid, and the synthetic 7,12- or isodeoxycholic acid) yield a series of respective choladienic acids, while cholic acid is dehydrated to cholatrienic acid (m. p. 163–164°C.), accompanied by several isomers in minor amounts (173). All these acids can be hydrogenated catalytically to the mother substance cholanic acid, which in fact was obtained for the first time via cholatrienic acid (199, 201).

Milder dehydrating agents like zinc chloride, concentrated hydrochloric acid, or potassium bisulfate lead by partial dehydration to unsaturated hydroxy acids such as dihydroxycholenic acids or hydroxycholadienic acids. The most important representatives of this type of compounds are 3,12-dihydroxycholenic acid and apocholic acid. Both were obtained simultaneously by Boedecker, and the former was transformed by catalytic hydrogenation into deoxycholic acid according to Boedecker's intention (17). This was the first transition from cholic acid to its most important companion acid. Claims of biochemical reduction of cholic acid to deoxycholic acid by microörganisms have never been experimentally substantiated. The difficulty of explaining the isomerism between dihydroxycholenic acid and apocholic acid led into various blind alleys until it was solved through the establishment of the new formula. Apocholic acid reacts with bromine and other oxidizing agents, but, although the presence of a double bond is betrayed by a yellow coloration with tetranitromethane, it displays absolute resistance against hydrogenation. This behavior finds a most plausible explanation (233) in the assumption that the double bond has migrated from $\Delta^{7,8}$ to $\Delta^{8,9}$ or $\Delta^{8,14}$. This view is supported by the analogy of the formation of $\Delta^{9,10}$-octalene (octahydronaphthalene) from α-decalol (97). This octalene likewise escapes hydrogenation and the same holds for hexadecahydrochrysene (28). This gradation in affinity towards hydrogen finds its counterpart in the stepwise dehydrogenation with selenium discussed previously (156; cf. 105).

Ergosterol through a peculiar dimeric intermediary compound may be converted into neoergosterol, $C_{27}H_{39}OH$ (99). While its formula calls for

four double bonds, only one can be detected by oxidation with benzoyl peroxide or catalytic hydrogenation; the other three double bonds have entered into a stable aromatic configuration presumably in ring II, as supported by the identification of 1,2,3,4-benzenetetracarboxylic acid, a remnant of its destructive oxidation with nitric acid. Toluenetetracarboxylic acid has been obtained by nitric acid oxidation of ergosterol itself. The methane originating by the cleavage of CH_3 and H from vicinal carbon atoms has been recovered by Inhoffen. This aromatization through loss of a methyl group follows the model of ionene (XXXII), which is converted by heating with sulfur into 3,8-dimethylnaphthalene (XXXIII) (163).



XXXII
Ionene,
$C_{13}H_{18}$

XXXIII
Dimethylnaphthalene,
$C_{12}H_{12}$

Tendency to aromatization through loss of CH_3H is characteristic for apocholic acid and its derivatives (233). Considerable amounts of methane are recovered when such substances are heated to 320–340°C. in an inert atmosphere such as carbon dioxide.

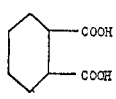
Cruder methods of dehydration lead to the formation of anhydrides and to polymerization of bile acids to amorphous insoluble masses long known as "dyslysins." The dehydration of keto acids gives rise to condensations between the carbonyl group of one molecule with a reactive methylene group of another molecule, but the substances mentioned in this paragraph are without significance for structural questions.

D. Thermic cyclization

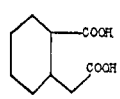
Dehydration by milder methods of keto and other polycarboxylic acids derived from bile acids, takes an important place in constitutional analysis of the carbon skeleton and the position of hydroxyl groups. The interpretation of these reactions is based on two rules borrowed from simpler model reactions, the so-called Blanc's rule and Bredt's rule.

Blanc (15) observed that glutaric acid on treatment with acetic anhydride forms an intramolecular anhydride, while adipic and pimelic acids subjected to the same treatment are converted into cyclopentanone and cyclohexanone, respectively (cf. 8). This is only an example of the well-known tendency of five- and six-membered rings to form whenever cy-

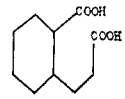
clization occurs. Blanc's experience was established as a "rule" by the German investigators, and they noticed that similar cyclizations can be frequently accomplished without the use of acetic anhydride by mere application of heat in a high vacuum. This rule can be applied to a great many substitution products of aliphatic dicarboxylic acids, e.g., in the terpene series to the formation of camphononic acid from homocamphoronic acid (118). Windaus, Hückel, and Reverey (256) studied the cyclization of hydroaromatic dicarboxylic acids, such as hexahydro-*o*-phthalic acid (XXXIV), hexahydrohomophthalic acid (XXXV), and hexahydro-*o*-carboxyhydrocinnamic acid (XXXVI) in the presence of acetic anhydride. The two former behave like succinic and glutaric acid, yielding anhydrides (XXXVII, XXXVIII) with five- and six-membered rings, while the cyclohexane-1-carboxylic acid-2-propionic acid (XXXVI) is transformed into hexahydro-α-hydrindone (XXXIX).



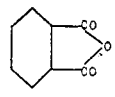
XXXIV
Hexahydro-*o*-phthalic acid, $C_8H_{12}O_4$



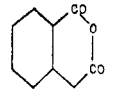
XXXV
Hexahydro-homophthalic acid, $C_9H_{14}O_4$



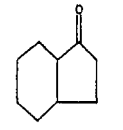
XXXVI
Hexahydro-*o*-carboxyhydrocinnamic acid, $C_{10}H_{16}O_4$



XXXVII
Anhydride of XXXIV, $C_8H_{10}O_3$

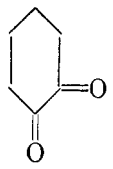


XXXVIII
Anhydride of XXXV, $C_9H_{12}O_3$

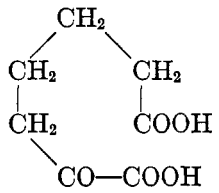


XXXIX
Hexahydro-α-hydrindone, $C_9H_{14}O$

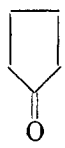
Adickes in Wieland's laboratory studied thermic reactions of α-ketopimelic and α-ketosuberic acids (4). The tendency towards the formation of cyclic diketones by loss of carbon dioxide and water by heating in nitrogen was greater in the case of ketopimelic acid (XL) $\rightarrow$ cyclohexanedione (XLI) than for ketosuberic acid (XLIII) $\rightarrow$ cycloheptanedione (XLIV). On the other hand, the reaction involving the loss of carbon dioxide, carbon monoxide, and water in the presence of concentrated sulfuric acid is realized to a higher percentage of the theoretical value in ketosuberic acid yielding cyclohexanone (XLV) than with the lower homolog (XLII). Thus in any event, reactions leading to the formation of six-membered rings are most prone to occur.



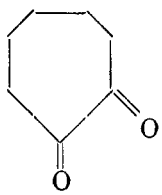
XLI
Cyclohexanedione,
$C_6H_8O_2$



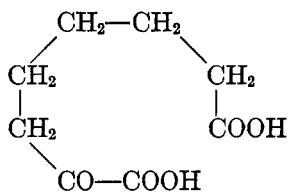
XL
Ketopimelic acid,
$C_7H_{10}O_5$



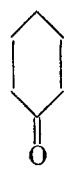
XLII
Cyclopentanone,
C_5H_8O



XLIV
Cycloheptanedione,
$C_7H_{10}O_2$



XLIII
Ketosuberic acid,
$C_8H_{12}O_5$

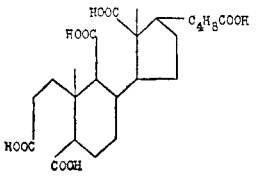


XLV
Cyclohexanone,
$C_6H_{10}O$

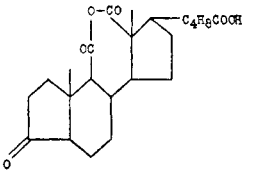
From these observations, the following general rule was formulated: Formation of anhydrides that can be "saponified" to the original acid with greater or lesser ease speaks for a 1,4- or 1,5-position of the participating groups. Cyclic ketones originate from 1,6-, 1,7- and possibly 1,8-dicarboxylic acids by loss of carbon dioxide and water. That one of two carboxyl groups in the 1,3-position will be expelled at moderately elevated temperature, is to be expected from substituted malonic acids.

As such ketonic ring structures may be opened by oxidizing agents as outlined in pp. 315 to 318, oxidation alternating with thermic cyclization allows stepwise degradation as a general method of structural investigation. The great usefulness of this method and the agreement of its results with conceptions derived from other evidence led to a general acceptance of the above interpretation. This entailed severe misconceptions in those cases where the diagnostic value of the method was founded on analogy only. These errors were recognized by Rosenheim and King, who pointed out that the formation of anhydrides by thermic cyclization does not in every case exclude 1,6-position of the carboxyl groups involved. The anhydride formation, in substances like choloidanic acid (XLVI → XLVII) or the isomer of lithobilianic acid, with cleavage of ring III (XCII → XLVIII) led Windaus, Wieland, and others to the assumption that anhy-

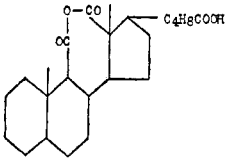
dride formation is adequate proof for the 1,5-position of the two carboxyl groups involved. Thus the ring, now designated as ring III, was assumed to be a five-membered ring which, because of the obvious presence of —CHOH—CH_2— or —CO—CH_2, could contain but three tertiary carbon atoms. Thilobilianic acid likewise forms an anhydride instead of a ketone, although ring II is six-membered (XCI → XLIX).



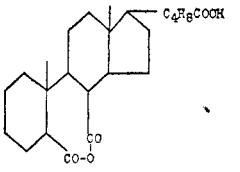
XLVI
Choloidanic acid, $C_{24}H_{36}O_{10}$



XLVII
Anhydride of pyrocholoidanic acid, $C_{23}H_{32}O_6$



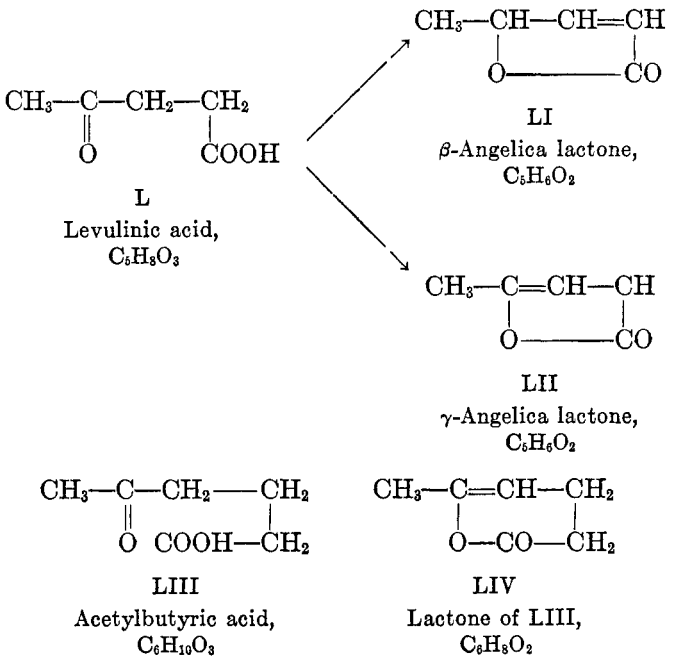
XLVIII
Anhydride of acid XCII, $C_{24}H_{36}O_8$



XLIX
Anhydride of thilobilianic acid (XCI), $C_{24}H_{36}O_5$

This in turn distracted attention from phenanthrene or chrysene systems as structural possibilities. An analogy in the aromatic series was unfortunately overlooked: diphenic acid forms an anhydride at 270°C. and no trace of the yellow fluorenone can be discerned in the distillate. This ketone forms, however, above 320°C. Hydroaromatic derivatives of diphenic acid have not yet been investigated, but it seems probable that the formation of seven-membered anhydride rings involves less distortion than that of five-membered ketone cycles.

The other regularity frequently encountered in this field pertains to the thermic cyclization of γ- and δ-keto acids, giving rise to the formation of unsaturated lactones of the angelica lactone type. Windaus and Bohne (262) devoted a very comprehensive study to such unsaturated lactones of aliphatic and terpenoid character and deduced the following rules: (a) The carbonyl group must be in the γ- or δ-position relative to the carboxyl group (L to LIV).



L
Levulinic acid,
$C_5H_8O_3$

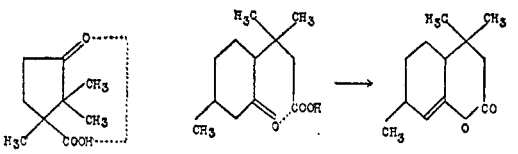
LI
β-Angelica lactone,
$C_5H_6O_2$

LII
γ-Angelica lactone,
$C_5H_6O_2$

LIII
Acetylbutyric acid,
$C_6H_{10}O_3$

LIV
Lactone of LIII,
$C_6H_8O_2$

(b) There must be a hydrogen atom attached to the α-carbon, i.e., the one next to the carboxyl group. (c) The keto group, when a member of a ring, must be in the α-position to that carbon atom of the ring from which the carboxyl-containing side chain starts. Otherwise, the present ring and the newly formed lactone ring would share three carbon atoms. As the double bond must be situated either in the α, β- or in the β, γ-position relative to the carbonyl group, this would lead to a conflict with the so-called Bredt rule. Bredt (29) observed that in terpenes with a 1,3-bridge, i.e., with two rings sharing three carbon atoms, no double bond could issue from either of the two pivotal atoms. Such acids, e.g., camphononic acid (LV), volatilize without decomposition, while the related pulegoneacetic acid is easily converted into an unsaturated lactone (LVI → LVII) (193).



LV
Camphononic acid (forms no lactone), $C_9H_{14}O_3$

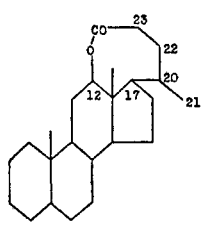
LVI
Pulegoneacetic acid, $C_{12}H_{20}O_3$

LVII
Lactone of LVI, $C_{12}H_{18}O_2$

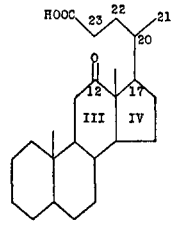
(d) The participation of the carboxylic side chain in a second cyclic structure also may cause steric hindrance against lactonization. (e) In polycarboxylic acids, reactions discussed previously as customary between pairs of carboxyl groups may successfully compete with lactonization. Thus, hyodeoxybilianic acid forms an unsaturated lactone at 240–275°C., while temperatures above 300°C. foster the synthesis of a saturated pyroketone.

The newly formed ring in such unsaturated lactones, as well as in the various pyro derivatives of the anhydride and the cyclanone type, usually corresponds to one of the original rings present in the hydroxy and dehydro derivative. But there are instances where one of the two ring fragments will undergo cyclization with the fragment of another ring. Examples of such "synthetic rings" may be found in the case of pyrobiloidanic acid, (CXXIX, CXXX on p. 343) and pyrocholoidanic acid (CVIII and CIX on p. 339), which form unsaturated lactones. The cyclopentanone ring in the acid $C_{15}H_{22}O_5$ (VI), pp. 316 and 345) is formed from fragments of rings II and III which were left over in acid $C_{16}H_{24}O_8$ (CXXXI, p. 344).

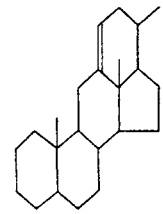
Other synthetic rings were observed in the formation of saturated lactones in the biliobanic series (see p. 337). Even the carboxyl group of the side chain may become involved, as exemplified in the lactonization of 12-hydroxycholanic acid (LVIII) (219). Similar lactonizations seem to occur to a limited extent with polyhydroxycholanic acids as deoxycholic acid. Intramolecular condensation between carbonyl in C_{12} and a methylene group of the side chain leads also to the formation of a fifth ring spanning from C_{17} in IV to C_{12} in III in the case of dehydronorcholene (LX) from 12-ketocholanic acid (XLIX).



LVIII
12-Hydroxycholanic lactone, $C_{24}H_{38}O_2$



LIX
12-Ketocholanic acid, $C_{24}H_{38}O_3$

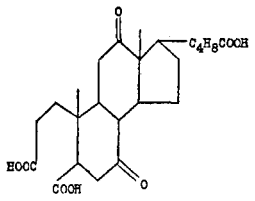


LX
Dehydronorcholene, $C_{23}H_{36}$

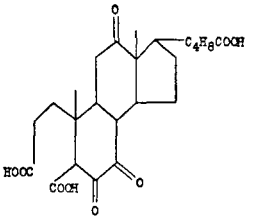
E. Other reactions of keto groups

The oxidation of a methylene group attached to a carbonyl group by permanganate results in the formation of a 1,2-diketone. These diketo substances easily undergo a rearrangement of the benzilic acid type in the presence of sulfuric acid, yielding a cyclic α-hydroxy acid, while the number

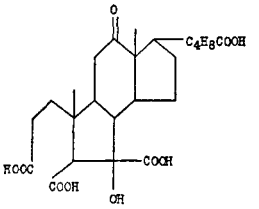
of carbon atoms within the ring is reduced by one. Thus, bilianic acid (LXI) is converted (LXII) into cilianic acid (LXIII) (22, 23).



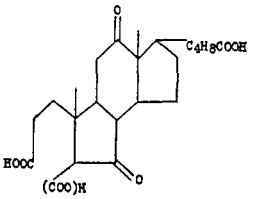
LXI
Bilianic acid,
$C_{24}H_{34}O_8$



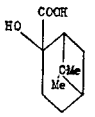
LXII
Triketo acid,
$C_{24}H_{32}O_9$



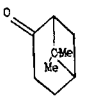
LXIII
Cilianic acid,
$C_{24}H_{34}O_{10}$



LXIV
Ciloxanic acid,
$C_{22}H_{32}O_6$



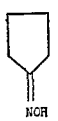
LXV
Nopinic acid,
$C_{11}H_{16}O_3$



LXVI
Nopinone,
$C_{10}H_{14}O$

For one of the reactions of cilianic acid, we may again choose a model reaction from Wallach's extensive researches in the terpene group. Nopinic acid (LXV) loses carbon monoxide and water when treated with permanganate in sulfuric acid and a cyclic ketone, nopinone (LXVI), is obtained (197, see also p. 318). Analogously, cilianic acid (LXIII) may be converted into ciloxanic acid (LXIV) via an unstable β-keto acid which is decarboxylated in the course of its formation (24). The cyclopentanone ring of this acid is identical with that obtained by thermic cyclization of 6,7-dicarboxyl derivatives.

Other experimental attempts to investigate the nature of rings containing carbonyl followed Wallach's (195) application of the Beckmann oxime rearrangement to cyclic ketoximes (LXVII to LXIX).

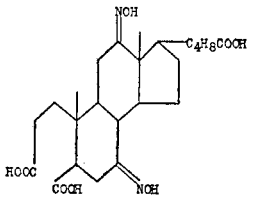


LXVII
Cyclopentanone oxime,
C_5H_9ON

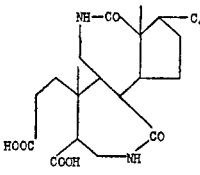
LXVIII
Cyclopentanone isoöxime,
C_5H_9ON
(lactam of LXIX)

LXIX
δ-Aminovaleric acid,
$C_5H_{11}O_2N$

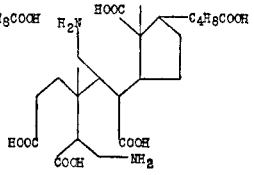
Hydroxylamine derivatives of dehydro bile acids were investigated in numerous cases by Schenck (166). The following compounds written in the modern formulation exemplify this rearrangement in the case of bilianic acid dioxime (LXX) → diisoöxime (LXXI), which can be considered as a double lactam of a diaminopentacarboxylic acid (LXXII). The chemistry of these nitrogenous derivatives corroborates the findings by the methods previously discussed.



LXX
Bilianic acid dioxime, $C_{24}H_{36}O_8N_2$



LXXI
Diisoöxime, $C_{24}H_{36}O_8N_2$



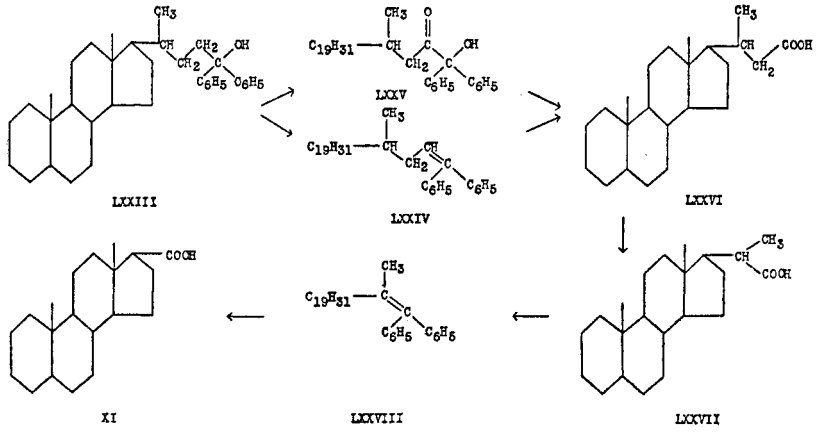
LXXII
Diaminopentacarboxylic acid, $C_{24}H_{40}O_{10}N_2$

E. Reduction of carboxyl groups

Reduction of carboxyl groups formed by oxidative cleavage was investigated by Wieland (220) in the model compound hexahydrophthalic acid. Reduction with sodium and amyl alcohol according to Bouveault yielded the glycol and its furanoid oxide. Neither this reaction nor the conversion of hexahydrophthalic acid dimethyl ester by methylmagnesium iodide into the double dimethylcarbinol promised new insight when applied to bile acid derivatives.

A succession of Grignard reactions proved, however, a very valuable tool in the gradual destruction of the side chain, which was studied in great detail for the case of cholanic acid by Wieland and his coworkers (221). The ethyl ester of this acid is converted by the appropriate Grignard reagent to the diphenyl-, dimethyl-, or diisopropyl- (cf. p. 348) carbinol. Such carbinols (LXXIII) yield on dehydration an unsaturated hydrocarbon (LXXIV) and on oxidation a ketocarbinol (LXXV). Both these derivatives, as well as the carbinol itself, can be oxidized with chromium trioxide to norcholanic acid (LXXVI). This lower homolog is converted through an analogous set of reactions into the next homolog bisnorcholanic acid (LXXVII). A third application of these methods leads to the unsaturated hydrocarbon diphenylbisnorcholene (LXXVIII). The oxidation of this substance brings about the simultaneous loss of two more carbon atoms. The resultant substance is called aetiocholanic acid (XI). A fourth Grignard reaction applied to this substance leads to diphenylaetiocholene and, by oxidation, to a dicarboxylic acid, indicating that the nineteenth carbon atom is a member of a ring. This acid is aetiobilianic

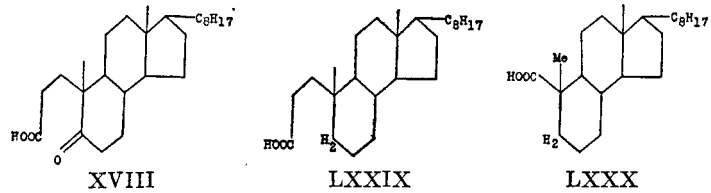
acid, and the fact that it forms an anhydride by high vacuum distillation would indicate, according to "Blanc's rule," that this ring is a five-membered one.



LXXIII. Norcholanyldiphenylcarbinol, $C_{36}H_{50}O$
LXXIV. Diphenylcholene, $C_{36}H_{48}$
LXXV. Ketocarbinol, $C_{36}H_{48}O_2$
LXXVI. Norcholanic acid, $C_{23}H_{38}O_2$
LXXVII. Bisnorcholanic acid, $C_{22}H_{36}O_2$
LXXVIII. Diphenylbisnorcholene, $C_{32}H_{44}$
XI. Aetiocholanic acid, $C_{20}H_{32}O_2$

This ring IV, which carries the side chain, is the one most resistant to any method of attack, since it never carries a hydroxyl group. Thus, oxidative methods will open up one, two, or all three of the other rings that carry hydroxyl groups in the naturally occurring bile acids, but ring IV will remain intact.

A similar series of Grignard reactions was applied by Tschesche (185) in the degradation of the keto acid (XVIII), $C_{26}H_{44}O_3$, obtained from coprostenone (XV). After reduction of the keto group to methylene (LXXIX), carbon atoms C_3 and C_2 were successively removed. The formation and the properties of the resulting acid (LXXX), $C_{24}H_{42}O_2$, corroborate the formula of Rosenheim and King. The great difficulty with which this acid is esterified supports the assumption that carbon C_{10} carries a methyl group (C_{18}) besides the carboxyl group of C_1.



XVIII
Keto acid,
$C_{26}H_{44}O_3$

LXXIX
Acid,
$C_{26}H_{46}O_2$

LXXX
Acid,
$C_{24}H_{42}O_2$

Grignard degradation of the side chain in a monocyclic (ring IV) acid will be further discussed below (p. 344).

IV. SYSTEMATIC DESCRIPTION OF BILE ACIDS AND THEIR DEGRADATION PRODUCTS

A. Hydroxycholanic acids

At least seven hydroxylated cholanic acids have been isolated from the bile of various animals; several others have been prepared synthetically.

Lithocholic acid, 3-hydroxycholanic acid, was first isolated by H. Fischer (65) from ox gall-stones; it is a normal constituent of ox bile and human bile. One gram could be isolated by Wieland (206) from 100 liters of ox bile which contained 5–6 kg. of cholic acid and 600–800 g. of deoxycholic acid. Lithocholic acid occurs presumably in a conjugated state like the other bile acids, although the actual isolation of its conjugated derivatives seems well-nigh impossible. It melts at 186°C.; its $[\alpha]_D$ is about + 32°. It has a tendency to appear in gelatinous form. It may be separated from deoxycholic acid by its lesser acidity, as deoxycholic acid will migrate into an ethereal layer from a slightly acid aqueous solution, leaving the monohydroxy acid behind.

By far the most important of the dihydroxy acids is deoxycholic acid, 3,12-dihydroxycholanic acid. Its similarity to the synthetic apocholic acid, 3,12-dihydroxycholenic acid, will be further discussed below (p. 360). When free of any retained solvent, it melts at 176.5°C. (corrected); its $[\alpha]_D$ is + 55°. For its purification see Sobotka and Goldberg (175).

Isomeric dihydroxy acids are the 3,6-dihydroxy acid, hyodeoxycholic acid, m.p. 196–197°C., found in hog bile (85, 257, 262, 263), and the 3,7-dihydroxy acid, chenodeoxycholic acid (or anthropodeoxycholic acid), m.p. 108°C., $[\alpha]_D = +11°$, in goose bile (129, 259) and in human bile (217). 7,12-Dihydroxycholanic acid, usually referred to as isodeoxycholic acid, is a synthetic product, melting at 226–227°C. (213).

3, 7,12-Trihydroxycholanic acid, or cholic acid, is present in the bile of all animals. It melts at 195°C.; its $[\alpha]_D = +37°$. Its isomer, 3,7,23-trihydroxycholanic acid or β-phocaecholic acid, was found by Hammarsten in the bile of the seal (88); its constitution has been established by Windaus and Van Schoor (264). It melts at 22°C.; its $[\alpha]_D = +27°$ to $+29°$.

3,7,8,12-Tetrahydroxycholanic acid (CXXI) (p. 341) was obtained by oxidation with permanganate from 3,12-dihydroxycholenic acid (26, 228). The natural occurrence of an isomeric tetrahydroxy acid is suspected, but not proven, in rabbit bile (264).

B. Ketocholanic acids and ketohydroxycholanic acids

The preparation and general reactions of the keto derivatives have already been discussed. The "dehydro derivatives" of all the hydroxy

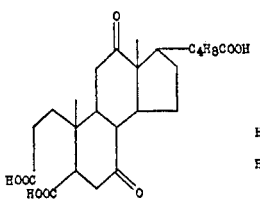
acids mentioned have been prepared, as well as partly oxidized derivatives containing both hydroxyl and keto groups. The 3-hydroxy-12-ketocholanic acid, m.p. 160°C., $[\alpha]_D = +111°$, prepared by Cerecedo (33) from 3-monoacetyldeoxycholic acid by oxidation, occurs in human bile associated with an equivalent amount of chenodeoxycholic acid. This molecular compound, melting at 165°C., was first isolated by Weyland in Wieland's laboratory; its constitution was determined by Wieland and Kishi (234), who separated the hydroxyketo acid in the form of its semicarbazone.

C. Acids with three rings

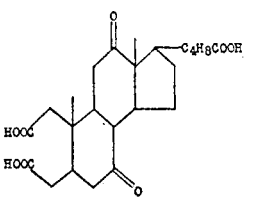
The simplest bile acid derivative containing three rings is the tricarboxylic acid, lithobilianic acid (LXXXIX), m.p. 275°C. (232), obtained by oxidation from dehydrolithocholic acid, where its formation is accompanied by that of isolithobilianic acid (XC). Other isomers obtained from the corresponding monoketo acids are thilobilianic acid (XCI) through cleavage of ring II between C_6 and C_7 (229) and another one (XCII) through cleavage of ring III between C_{11} and C_{12} (206, 209), all three isomers melting at about 260–262°C. Aetiobilianic acid, melting at 228°C. with opening of ring IV, after loss of the side chain, has already been mentioned (p. 317). Butenandt, Weidlich, and Thompson (31) recently obtained a dimethylphenanthrene, melting at 140°C., from aetiobilianic acid (XIV) by dehydrogenation and simultaneous double decarboxylation with selenium. An identical product was obtained from the hydrate of follicular hormone (CLIX), and its constitution as 1,2-dimethylphenanthrene was proved by Haworth's synthesis (91) (cf. p. 353).

Lithobilianic acid and isolithobilianic acid are the parent substances of a number of ketotricarboxylic acids derived from the various natural bile acids through their dehydro derivatives by further oxidation which attacks ring I whenever it contains oxygen on C_3. The acids obtained are bilianic acid (LXI), or 7,12-diketolithobilianic acid, m.p. 275°C., $[\alpha]_D = +76°$, and isobilianic acid (LXXXII), or 7,12-diketoisolithobilianic acid, m.p. 234–237°C. (115), substances whose isolation was one of the first achievements of the oxidative degradation of cholic acid. Deoxybilianic acid (LXXXIII), or 12-ketolithobilianic acid, m.p. 296°C., $[\alpha]_D = +94°$ (204, 223, 225), was discovered likewise by Lachinov (116), who called this acid "cholanic acid," a term abandoned later since it was applied to the mother substance of the bile acids (cf. 203). As well as its isomer, isodeoxybilianic acid (LXXXIV), Lachinov's "isocholanic acid" of m.p. 247–248°C. and $[\alpha]_D = +73°$, it is derived by oxidation from deoxycholic acid or by reduction, with zinc and fuming hydrochloric acid (21), of the more reactive

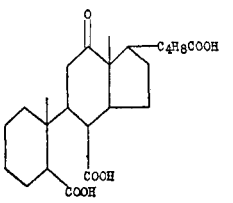
group C_7 in bilianic and isobilianic acids, respectively. Chenodeoxybilianic acid (LXXXV), m.p. 230°C. (263), or 7-ketolithobilianic acid, is very easily reduced by Clemmensen's method to lithobilianic acid. Hyodeoxybilianic acid (LXXXVI), or 6-ketolithobilianic acid, has never been isolated on account of its β-keto group, which causes immediate decarboxylation to a ketodicarboxylic acid (LXXXVII). Hyoisodeoxybilianic acid exists only in its stereomeric or "allo"-form and will be discussed below (pp. 349, 350) under the name keto-Staden acid. The oxidation product of 7,12-diketocholanic acid, dehydroisodeoxycholic acid, is known as pseudodeoxybilianic acid (LXXXVIII) m.p. 259–260°C. (21). Further oxidation of this acid leads to a pentacarboxylic acid discussed in the following section.



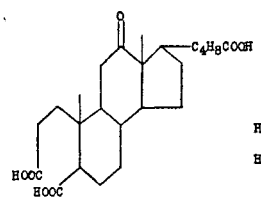
LXI
Bilianic acid,
$C_{24}H_{34}O_8$



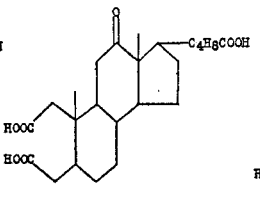
LXXXII
Isobilianic acid,
$C_{24}H_{34}O_8$



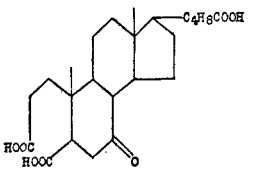
LXXXVIII
Pseudodeoxybilianic acid,
$C_{24}H_{36}O_7$



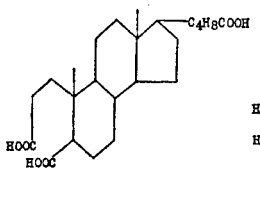
LXXXIII
Deoxybilianic acid,
$C_{24}H_{36}O_7$



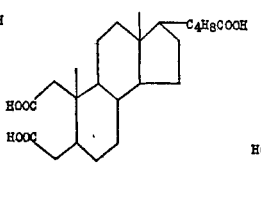
LXXXIV
Isodeoxybilianic acid,
$C_{24}H_{36}O_7$



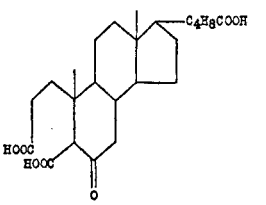
LXXXV
Chenodeoxybilianic acid,
$C_{24}H_{36}O_7$



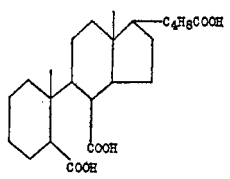
LXXXIX
Lithobilianic acid,
$C_{24}H_{38}O_6$



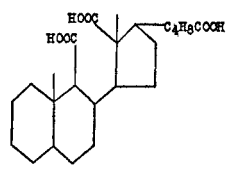
XC
Isolithobilianic acid,
$C_{24}H_{38}O_6$



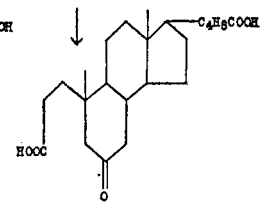
LXXXVI
(Hyodeoxybilianic acid,
$C_{24}H_{36}O_7$)



XCI
Thilobilianic acid,
$C_{24}H_{38}O_6$



XCII
Isomeric acid,
$C_{24}H_{38}O_6$



LXXXVII
Ketodicarboxylic acid,
$C_{23}H_{34}O_5$

Either oxidation of cholic acid with hypobromite or catalytic reduction of bilianic acid leads to 7-hydroxy-12-ketolithobilianic acid. The carboxyl group in C_4 forms a γ-lactone with the hydroxyl group at C_7. This acid of melting point 303–304°C. (212, 22) was first prepared by Pringsheim (144), who gave it the name biliobanic acid (V) (p. 315). By further catalytic reduction, reductobiliobanic acid (XCIII), or 7,12-dihydroxylithobilianic acid, m.p. 243°C., can be prepared (25). The isomeric isobiliobanic acid (XCV), melting at 264°C., is the ϵ-lactone of 12-hydroxy-7-ketoisolithobilianic acid. It cannot be prepared, however, from isobilianic acid (LXXXII, p. 335), as we know of no method that would reduce the carbonyl in C_{12} without reducing that in C_7. It can only be obtained by reduction of both keto groups in isobilianic acid to reductoisobiliobanic acid (LXXXIV), or 7,12-dihydroxyisolithobilianic acid, and subsequent partial oxidation of the $\rangle$CHOH in position C_7.

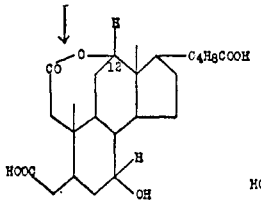
Another interesting substance of this type is chenodeoxybiliobanic acid (XCVI), obtained by Clemmensen reduction of the keto group at C_{12} in biliobanic acid, or by the action of hypobromite upon chenodeoxycholic acid (XCVII) (261, 222). The lactone melts at 253°C., the free acid at 255–256°C. (22).

Thermic reclosure of ring I in the bilianic acid group leads to a number of pyrobilianic acids, all of which can be derived from pyrolithobilianic acid (XCVIII) and pyroisolithobilianic acid (XCIX). They contain four rings. Oxidative opening of this new cyclopentanone ring results in the formation of norbilianic acids, such as nordeoxybilianic acid (C), melting at 232–233°C. (210).

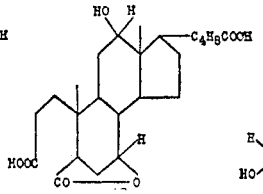
Oxidation of pyrodeoxybilianic acid (CI) with permanganate leads to a diketodicarboxylic acid (CII), $C_{23}H_{34}O_6$, m.p. 216°C. (204). This tricyclic acid, being relatively accessible, was made the starting point for several investigations by Wieland (215, 227). On further oxidation with permanganate, norcilianic acid (CIII) is obtained, a bicyclic diketotetracarboxylic acid melting at 122°C.

Oxidation of bilianic acid (LXI) with permanganate leads to a hypo-

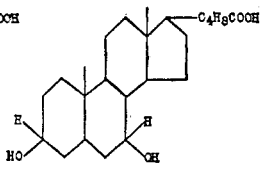
LXXXII
Isobilianic acid,
(see p. 335)



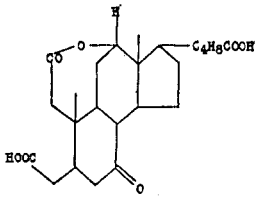
XCIV
Reductoisobiliobanic acid,
$C_{24}H_{38}O_7$



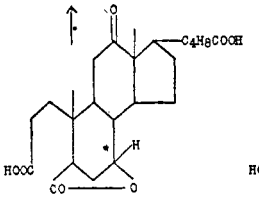
XCIII
Reductobiliobanic acid,
$C_{24}H_{38}O_7$



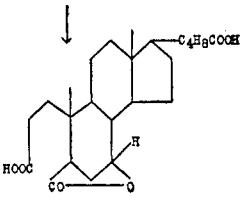
XCVII
Chenodeoxycholic acid,
$C_{24}H_{40}O_4$



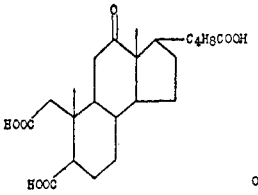
XCV
Isobiliobanic acid,
$C_{24}H_{36}O_7$



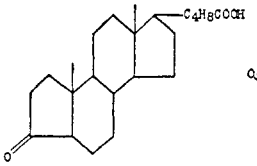
V
Biliobanic acid,
$C_{24}H_{36}O_7$



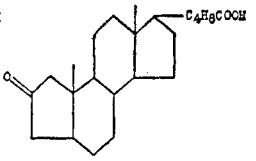
XCVI
(Cheno)deoxybiliobanic acid,
$C_{24}H_{38}O_6$



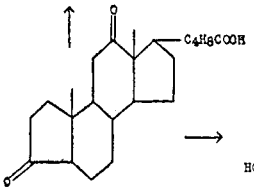
C
Nordeoxybilianic acid,
$C_{23}H_{34}O_7$



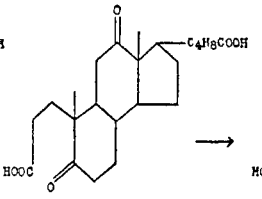
XCVIII
Pyrolithobilianic acid,
$C_{23}H_{36}O_3$



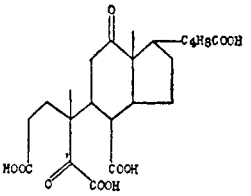
XCIX
Pyroisolithobilianic acid,
$C_{23}H_{36}O_3$



CI
Pyrodeoxybilianic acid,
$C_{23}H_{34}O_4$



CII
Wieland's diketodicarboxylic acid,
$C_{23}H_{34}O_6$



CIII
Norcilianic acid,
$C_{23}H_{32}O_{10}$

thetical 6,7,12-triketolithobilianic acid (LXII), which suffers a benzilic acid rearrangement to cilianic acid (LXIII); this in turn, as mentioned on p. 330, is converted by permanganate and sulfuric acid into ciloxanic acid (LXIV), m.p. 216–217°C. (24).

Further oxidation of bilianic acids and their derivatives leads to bicyclic acids.

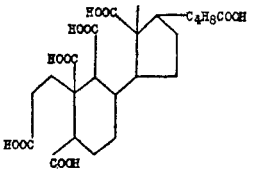
D. Acids with two rings

Just as lithobilianic acid is the simplest tricyclic bile acid derivative and may be considered, at least theoretically, as the mother substance of the bilianic acid group, so choloidanic acid is the prototype of bicyclic acids. It was first obtained by Cleve (36) from impure cholic acid, containing deoxycholic acid, by nitric acid oxidation. Lachinov (113) recognized deoxycholic acid as its only source (cf. reference 205), but was misled by certain similarities to camphoric acid and called the substance "cholecamphoric acid." It retains rings II and IV unaltered, while rings I and III have both been split to yield four carboxylic groups (XLVI) (see p. 327). M.p. $\simeq$ 300°C.; $[\alpha]_D = +40°$.

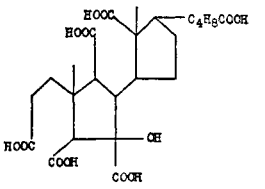
Closely related to choloidanic acid is chollepidanic acid (CIV), m.p. 280°C., which is obtained from deoxybilianic acid by strong oxidation with nitric acid. Its name is derived from the Greek word λεπις, lepis, meaning "scale," suggested by the appearance of its crystals. Its constitution is not completely elucidated, involvement of the side chain in the oxidative destruction may be ruled out, and Wieland assumes that the methyl group attached to C_{10} is oxidized to carboxyl. The acid is distinguished by its barium and calcium salts, which are less soluble in hot than in cold water (214, 223, 230).

Another acid in which rings I and III are disrupted is ciloidanic acid (CV), derived from cilianic acid (LXIII) by nitric acid oxidation. Its melting point is 248°C. (209). It bears the same relation to cilianic acid as choloidanic acid (XLVI, p. 327) does to deoxybilianic acid (LXXX, p. 335). In analogy with the transition of cilianic into ciloxanic acid (LXIV, p. 330), ciloidanic acid is converted into a ketopentacarboxylic acid (CVI) which, as a β-keto acid, is decarboxylated at once to a ketotetracarboxylic acid (CVII) melting at 238°C. (211).

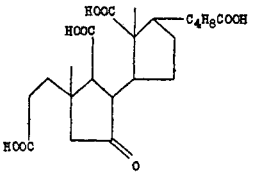
Thermic cyclization of ring I in choloidanic acid (XLVI, p. 327) yields pyrocholoidanic acid (CVIII). This acid has interesting properties, as it occurs in two stereomeric hydrated forms and as an unsaturated lactone. Inspection of a spatial model shows that lactonization probably takes place between the carboxyl in position C_{12} and the enolized carbonyl in C_3 (CIX). Its oxidation with permanganate proceeds analogously to the oxidation of pyrodeoxybilianic acid to Wieland's diketodicarboxylic acid



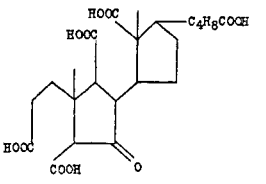
CIV
Chollepidanic acid,
$C_{24}H_{34}O_{12}$



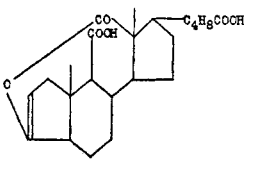
CV
Ciloidanic acid,
$C_{24}H_{34}O_{13}$



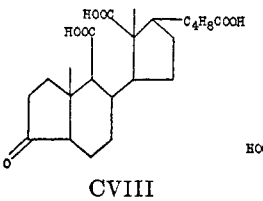
CVII
Ketotetracarboxylic acid,
$C_{22}H_{32}O_9$



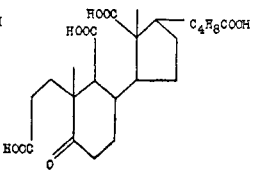
CVI
Ketopentacarboxylic acid,
$C_{23}H_{32}O_{11}$



CIX
Lactone of CVIII,
$C_{23}H_{32}O_6$



CVIII
Pyrocholoidanic acid,
$C_{23}H_{34}O_7$

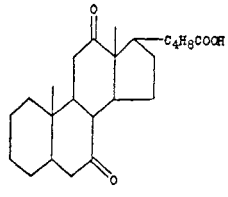


CX
Prosolannellic acid,
$C_{32}H_{34}O_9$

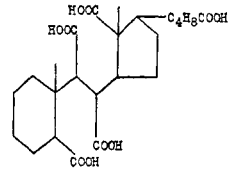
(CI, CII, p. 337). The resulting ketotetracarboxylic acid, m.p. 220°C., is designated prosolannellic acid (CX) for reasons cited below (207).

The number of bicyclic derivatives in which ring II is disrupted is much more limited. Rings II and III are opened in isocholoidanic acid (CXII), m.p. 273°C. (213), which is prepared from 7,12-diketocholanic acid (CXI) or the corresponding "pseudo" deoxybilianic acid (LXXXVIII) by treatment with nitric acid. Rings I and IV are intact and the carboxyl groups are in positions C_6, C_7, C_{11}, and C_{12} in addition to the original carboxyl in C_{24}.

A hydroxyl group was introduced by means of bromination and alkali into position C_{11} of 12-ketocholanic acid (CXIII), an isomer of dehydrolithocholic acid (231). Ring III was split by permanganate and the resulting keto acid (CXIV) was again hydroxylated on the tertiary carbon atom 8. The oxidation of this hydroxyketo acid to the ketotricarboxylic acid (CXV) of m.p. 196°C. is the first instance where the link C_8—C_9 has

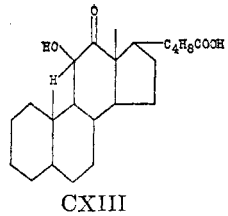


CXI
7,12-Diketocholanic acid,
$C_{24}H_{36}O_4$

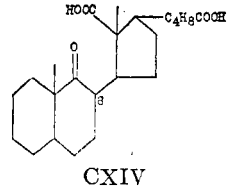


CXII
Isocholoidanic acid,
$C_{23}H_{36}O_{10}$

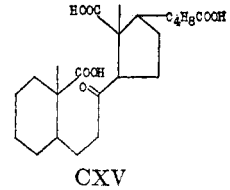
been disrupted while rings I and IV remain intact. Oxidation of this acid, $C_{23}H_{36}O_7$, with nitric acid (235) leads through simultaneous elimination of ten carbon atoms (Nos. 1–7, 9, 10, and 18) to the tricarboxylic acid, $C_{13}H_{20}O_6$, obtained previously by Wieland, Schlichting, and Vocke (216, 223).



CXIII
11-Hydroxy-12-keto-cholanic acid,
$C_{24}H_{38}O_4$



CXIV
Ketodicarboxylic acid,
$C_{23}H_{36}O_5$

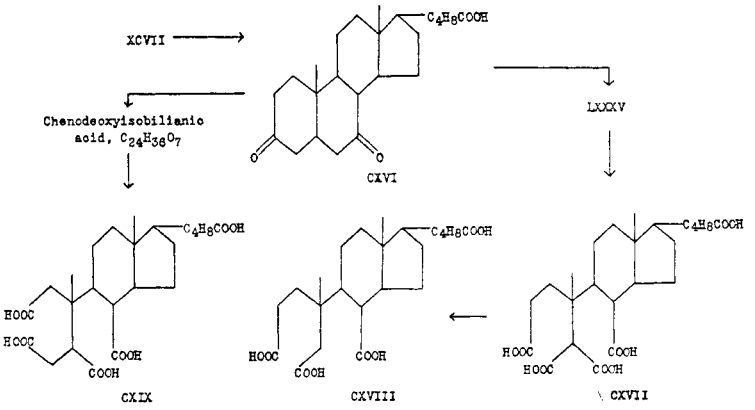


CXV
Ketotricarboxylic acid,
$C_{23}H_{36}O_7$

A third important isomer of choloidanic acid is chenocholoidanic acid (CXVII), m.p. 225°C., obtained from the corresponding dihydroxy- (XCVII), diketo- (CXVI), or bilianic (LXXXV) acid (218, 261). This acid with its carboxyl groups in positions 3, 4, 6, and 7 of the disrupted rings I and II, has two carboxyls attached to one carbon atom, i.e., C_6; because of this "malonic" character it is easily decarboxylated to a tetracarboxylic acid (CXVIII) of m.p. 219–220°C., while its isomer, chenoisocholoidanic acid (CXIX), m.p. 198–199°C. (218), with carboxyl groups in positions 2, 3, 6, 7, and 24, is stable.

The oxidation of $\Delta^{7,8}$-3,12-dihydroxycholenic acid (CXX) leads by way of 3,7,8,12-tetrahydroxycholanic acid (CXXI, (26) see p. 333) and 7,8-dihydroxy-12-ketolithobilianic acid (CXXII) to a diketotetracarboxylic acid (CXXIII), whose properties and constitution corroborate the assumption of the double bond between C_7 and C_8 in dihydroxycholenic acid (228, 233).

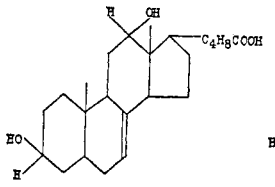
Bicyclic derivatives with rings IV and III open seem to be formed by oxidative destruction from the female sex hormone (124).



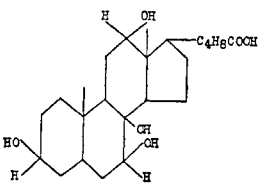
XCVII. Chenodeoxycholic acid (see p. 337)
CXVI. Dehydrochenodeoxycholic acid, $C_{24}H_{36}O_4$
LXXXV. Chenodeoxybilianic acid (see p. 335)
CXVII. Chenocholoidanic acid, $C_{24}H_{36}O_{10}$
CXVIII. Tetracarboxylic acid, $C_{23}H_{36}O_8$
CXIX. Chenoisocholoidanic acid, $C_{24}H_{36}O_{10}$

E. Acids with one ring

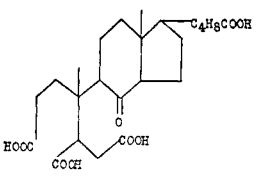
The scission of the three rings I, II, and III, would yield a heptacarboxylic acid of the formula CXXIV by analogy with lithobilianic acid,



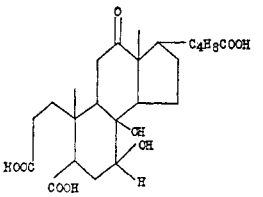
CXX
3,12-Dihydroxycholenic acid, $C_{24}H_{38}O_4$



CXXI
3,7,8,12-Tetrahydroxycholanic acid, $C_{24}H_{40}O_6$

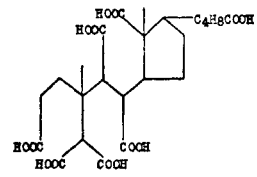


CXXIII
Diketotetracarboxylic acid, $C_{24}H_{34}O_{10}$



CXXII
7,8-Dihydroxy-12-ketolithobilianic acid, $C_{24}H_{36}O_9$

choloidanic acid, or chenocholoidanic acid. The close interrelationship between rings I and II, however, is not in favor of the accumulation of so



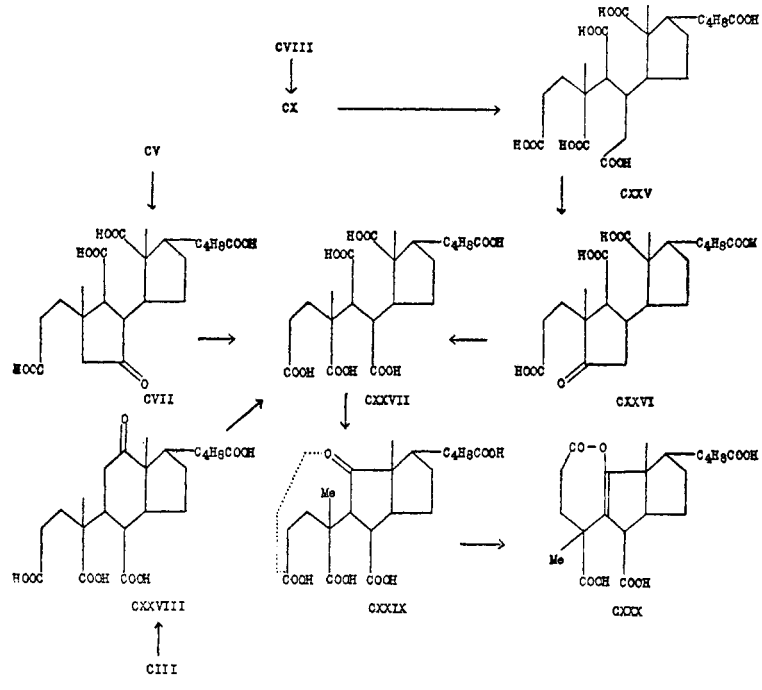
CXXIV

many carboxyl groups and, as a result, no monocyclic oxidation products containing the complete twenty-four carbon atom skeleton is known. Two lower homologs take its part in the scheme of oxidative degradation of bile acids: biloidanic acid (CXXVII), $C_{22}H_{32}O_{12}$, m.p. 226–228°C., first isolated by Letsche (119), and Wieland's solannellic acid (CXXV) (*solus annellus* = one ring only), $C_{23}H_{34}O_{12}$, m.p. 202–203°C. (207).

The latter acid is formed by oxidative scission of ring II, between the carbonyl in C_5 and the methylene in C_6 of prosolannellic acid (CX). This acid has been previously mentioned (p. 339), as an oxidation product of pyrocholoidanic acid. Solannellic acid was also obtained directly from pyrocholoidanic acid (CVIII). Ring II, which has been rebuilt as a cyclopentanone ring in pyrosolannellic acid, m.p. 272–273°C. (207), is reopened by nitric acid. The hexabasic acid formed was called norsolannellic acid by Wieland (211), but he soon established its identity with the well-known biloidanic acid, first isolated by Letsche and named by Schenck (166a). This hexabasic acid with twenty-two carbon atoms was prepared by the action of nitrating mixture (HNO_3 + H_2SO_4) upon cholic acid (119) and by the action of fuming nitric acid on cholic acid, bilianic acid, or pyrodeoxybilianic acid (CI) (207, 208). More perspicuous reactions resulting in the formation of biloidanic acid are (1) the oxidation of the ketotetracarboxylic acid (CVII), $C_{22}H_{32}O_9$, from ciloidanic acid (CV, p. 339), (2) the oxidation of the isomeric ketotetracarboxylic acid (CXXVIII), derived from norcilianic acid (CIII, p. 337) through loss of carbon monoxide, and (3) the oxidation of a third isomeric ketotetracarboxylic acid, namely, pyrosolannellic acid (CXXVI) as mentioned above.

Both solannellic acid and biloidanic acid are quite stable against further oxidation; the remnants of the three rings are disposed in such a manner that carboxyl groups are separated by at least two carbon atoms.

While thermic cyclization of solannellic acid leads to a cyclopentanone in place of ring II, biloidanic acid under "pyro" conditions fuses the carboxyl groups C_{11} and C_{12} into a keto group (CXXIX), which in turn forms an unsaturated lactone with carboxyl group C_3 (CXXX). The cyclopentanone structure of ring III in pyrobiloidanic acid is noteworthy,



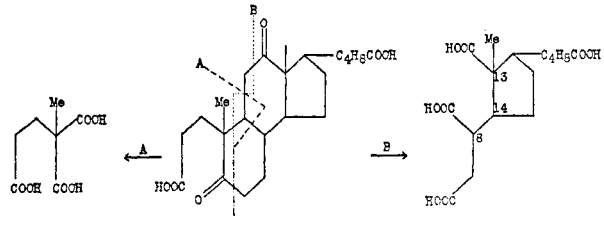
CIII. Norcilianic acid (p. 337)
CV. Ciloidanic acid (p. 339)
CVII. Ketotetracarboxylic acid (p. 339)
CVIII. Pyrocholoidanic acid (p. 339)
CX. Prosolannellic acid (p. 339)
CXXV. Solannellic acid, $C_{23}H_{34}O_{12}$
CXXVI. Pyrosolannellic acid, $C_{22}H_{32}O_9$
CXXVII. Biloidanic acid (= nor-solannellic acid), $C_{22}H_{32}O_{12}$
CXXVIII. Ketotetracarboxylic acid, $C_{22}H_{32}O_9$
CXXIX. Pyrobiloidanic acid, $C_{21}H_{30}O_9$
CXXX. Unsaturated lactone of CXXIX, $C_{21}H_{28}O_8$

since all other derivatives with carboxyl groups in C_{11} and C_{12} fail to behave as expected of 1,6-dicarboxylic acids, according to "Blanc's rule."

The various possibilities of partial and complete esterification in these polybasic acids deserve mention; they may be studied in the original publications, e.g., in the paper by Wieland and Schlichting on the various esters of biloidanic acid (208).

While most methods of oxidative degradation seem to be exhausted at this stage, oxidation of Wieland's diketodicarboxylic acid, $C_{23}H_{38}O_6$, leads further to the important tetracarboxylic acid (CXXXI), $C_{16}H_{24}O_8$, of m.p. 218°C. (216, 223, 224). This acid was first obtained as a by-product in the oxidation of pyrodeoxybilianic acid to Wieland's diketo acid. Better yields can be obtained by the use of nitrating mixture on the latter acid.

Yields of 14.5 per cent by weight of the starting material, deoxycholic acid, were reached by Wieland. The carboxyl group C_{12} behaves rather passively and this indicates the presence of a methyl group C_{19} attached to C_{13}. This assumption is further supported by the formation of acetone upon reaction with hydrogen peroxide, which finds a parallel in the case of α-methylglutaric acid or α-methyladipic acid (cf. p. 318). By-products in the preparation of this acid are succinic acid and α-methyl-α-carboxyglutaric acid (CXXXII) (= n-butane-1,3,3'-tricarboxylic acid (223, 192)). The simplest explanation of the appearance of these acids seems to be that indicated by the broken lines in formula CII in agreement with the assumption of a methyl group (C_{18}) on C_{10} (cf. reference 156). A green intermediary nitroso derivative indicates that the point of attack for the nitric acid (or rather for the nitrous acid) is the tertiary carbon atom C_9.



CXXXII
α-Methyl-α-carboxyglutaric acid, $C_7H_{10}O_6$

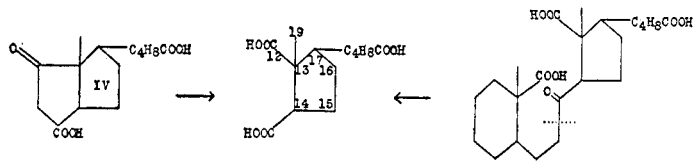
CII
Wieland's diketodicarboxylic acid, $C_{23}H_{34}O_6$

CXXXI
Tetracarboxylic acid, $C_{16}H_{24}O_8$

Thermic cyclization of the tetracarboxylic acid $C_{16}H_{24}O_8$ (CXXXI) leads to the ketodicarboxylic acid (VI), $C_{15}H_{22}O_5$, m.p. 187°C., in poor yields (7 g. from 145 g.). This pyro acid can be deprived of two more carbon atoms in two different series of reactions as follows: (a) Nitric acid or bromine (cf. p. 316) disrupt the cyclopentanone ring, and both lead to tricarboxylic acid (CXXXIII), $C_{13}H_{20}O_6$, also melting at 187°C., which was recently obtained by another route, namely from acid CXV (235). This acid forms an anhydride, and the carboxyl group on C_{13} displays the same inactivity as the analogous carboxyl group in $C_{16}H_{24}O_8$. (b) Reduction by Clemmensen's method to $C_{15}H_{24}O_4$ (CXXXIV) furnished a starting material for a Grignard reaction along the lines exemplified in the case of cholanic acid → norcholanic acid → bisnorcholanic acid. The carboxyl groups C_9 and C_{24} are converted into diphenylcarbinol groups. Whilst the former remains protected against oxidation, C_{24} and subsequently C_{23} can be eliminated (CXXXV, CXXXVI). Further progress was impossible because of the small amounts of material available (224).

The formation of the substances mentioned in the preceding paragraph

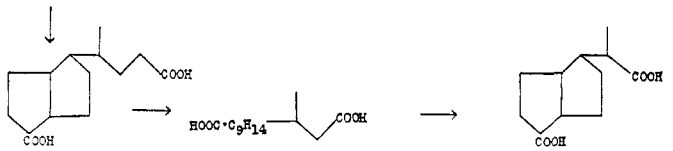
is in complete agreement with all other evidence and confirms decisively the conceptions developed during the last year. The five-membered nature of ring IV has been corroborated by the formation of dimethylphenanthrene from aetiobilianic acid (see pp. 334 and 353).



VI
Ketodicarboxylic acid, $C_{15}H_{22}O_5$

CXXXIII
Tricarboxylic acid, $C_{13}H_{20}O_6$

CXV
Ketotricarboxylic acid, $C_{23}H_{36}O_7$



CXXXIV
Dicarboxylic acid, $C_{15}H_{24}O_4$

CXXXV
"Nor" acid, $C_{14}H_{22}O_4$

CXXXVI
"Bisnor" acid, $C_{13}H_{20}O_4$

Conclusive evidence for the allocation of the methyl group C_{19} to C_{13} (and not C_{14}) lies in the steric configuration of the cyclopentanone ring in the pyro acid $C_{15}H_{22}O_5$. Its cleavage product, the tricarboxylic acid $C_{13}H_{20}O_6$ (CXXXIII), m.p. 187°C., bears the two carboxyl groups on C_{13} and C_{14} in the *trans*-position, since a lower melting, more soluble isomer (m.p. 137°C.) is obtained via the common anhydride. Thus the cyclopentanone ring in $C_{15}H_{22}O_5$, too, must have been attached in the *trans*-position. Ordinarily, such cycloketones would assume the *cis*-position, if necessary by an inversion. This rearrangement is however impossible in the parent substance $C_{16}H_{24}O_8$ (CXXXI, on pp. 343 to 344), if we assume that C_{13} carries no H, but the methyl group C_{19} (235).

Finally, the side chain can now be definitely allocated to C_{17} (instead of the alternative C_{15}), both because of consideration of molecular dimension and shape, and because of ring closures between carbonyl C_{12} and methylene C_{23} of the side chain, an example of which is given on p. 329 (156). The side chain is in the *cis*-position to the link C_{12}—C_{13}, i.e., in the *trans*-position relative to the methyl group, C_{19} (235).

F. Steric isomerisms

The study of the monocyclic derivatives discussed in the preceding section throws some light on the relative steric configuration of the asym-

metric carbon atoms C_{13}, C_{14}, and C_{17}. Similar considerations, e.g., of the pyro reactions of dihydro-Diels acid and its isomer (pp. 349, 350), let us assume that the cholanic acid–coprostane series bears the same relation to the allocholanic–cholestane series in regard to the relative position of rings I and II as *cis*-decalin does to *trans*-decalin. The steric relation between rings II and III is as yet unknown.

There is no more doubt, however, that the cyclic portion of the carbon skeleton is identical in these two large biological groups except for the steric configuration of one carbon atom, which can be only C_5, according to the formula of Rosenheim and King.

A glance at the formula of cholanic acid reveals eight asymmetric carbon atoms, viz., C_5 and C_{10}, C_8 and C_9, C_{13} and C_{14}, C_{17} and C_{20}. Even on the assumption that certain steric isomers are possible in theory only, but could not be realized because of excessive distortion of the ring system, it seems remarkable that sterols and bile acids should tally except for the constellation on a single carbon atom. This peculiarity reminds one of the unique prevalence of *d*-glucose among the numerous possible stereomeric aldohexoses.

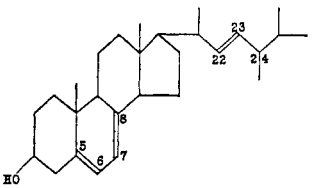
As laws of mutual induction seem to govern biosynthesis of the carbon skeleton, the position of the hydroxyl groups is likewise subject to a natural selection. While the secondary alcohol groups in positions C_3, C_6, C_7 and C_{12} create additional centers of asymmetry and offer opportunities for "epimerism," only one of the alternatives is ever realized in natural products. A few epimers have been artificially prepared in the sterol series by inversion of the —OH on C_3. Treatment with sodium in amyl alcohol catalyzes the establishment of equilibria between cholestanol, m.p. 141°C. (dihydrocholesterol, also β-cholestanol), and epicholestanol, m.p. 182°C. (ε-cholestanol (249)). Analogously, coprostanol (stercorin (66), coprosterol (20)) m.p. 99–100°C., had been converted in good yield into epicoprostanol, melting at 119°C., by Dorée and Gardner ("pseudocoprosterol" (55, 56), also δ-cholestanol (250, 251)). These epimers are distinguished from the natural sterols by their inability to form insoluble addition compounds with saponins (cf. p. 359).

G. Sterols with longer side chains

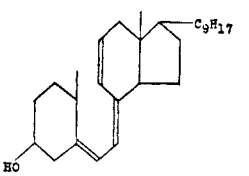
Ergosterol (CXXXVII), $C_{28}H_{43}OH$, m.p. 160°C., discovered by Tanret (182) in ergot, occurs in a variety of fungi, including yeast, where it is accompanied by zymosterol, m.p. 109°C. (122, 93, 149). Since ergosterol was recognized to be provitamin D, its chemistry has been studied extensively. It contains the same carbon skeleton as cholesterol, with an additional methyl group on C_{24}. Of its three double bonds, one is located in the side chain between C_{22} and C_{23} (formation of methylisopropylacetal-

dehyde with ozone (150, 82), see p. 319), the other two are in conjugation with each other in ring II (C_5=C_6, C_7=C_8) (159).

By ultra-violet irradiation of ergosterol, a number of isomers are formed, all of which respond to the formula of ergosterol, but none of which is precipitable with digitonin; they carry the names lumisterol, protachysterol (?), tachysterol, calciferol (crystallized vitamin D), toxisterol (?), suprasterol I and II. Only vitamin D has antirachitic properties. Lettré (269) has shown that tachysterol contains four double bonds, i.e., one more than ergosterol, and suggests that irradiation causes cleavage of ring II and simultaneous formation of a new double bond, by analogy with the photochemically induced cleavage of the cyclohexanone ring (XLV) to hexenaldehyde CH_2=$CH \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CHO$ (270). Formula CXXXVIII for tachysterol would explain the peculiarities of the irradiation products mentioned, their inability to combine with digitonin, and their failure to yield tetracyclic hydrocarbons on selenium dehydrogenation. Migration of the double bonds upon further irradiation is perhaps the mechanism underlying the formation of vitamin D and subsequently of the end products in which the conjugated system of double bonds no longer exists, thus abolishing further absorption of and sensitivity against ultra-violet



CXXXVII
Ergosterol, $C_{28}H_{43}OH$



CXXXVIII
Tachysterol, $C_{28}H_{43}OH$
(Lettré, 269)

rays. A number of other ergosterol isomers have been synthesized by purely chemical methods, but none of them could be identified with any of the irradiation products, nor did they display any vitamin D activity.

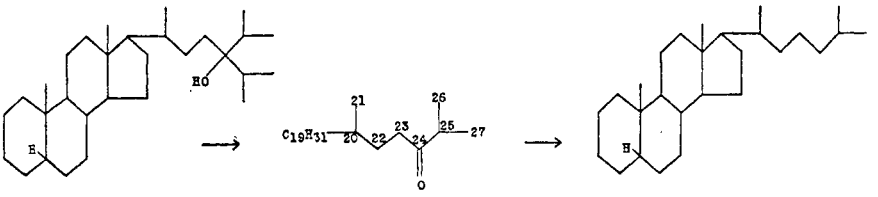
The best known representatives of the phytosterols, the sterols of higher plants, are sitosterol $C_{29}H_{49}OH$, m.p. 137°C. (σιτος, Greek, "wheat") and stigmasterol, $C_{29}H_{47}OH$, m.p. 170°C. (*Physostigma venenosum* = calabar bean). One of the two double bonds of the latter is located in the side chain as in ergosterol, but this sterol carries an *ethyl* group on C_{24}. The only double bond of sitosterol can be hydrogenated; the resulting saturated sterol, sitostanol, has likewise been found in nature. It is not identical, but is probably stereomeric, with stigmastanol, the tetrahydro derivative of stigmasterol (258, 6, 165, 64).

It may be mentioned in passing that certain saponins (amyrin, rhamnol, etc.) and cardiac poisons, as strophanthidin (101), resemble the sterols structurally and may be classified with them. Phytosterolins, glucosides of phytosterols, are well-defined substances and some have been synthesized.

Certain lower animals contain sterols different from cholesterol; spongosterol from sponges, asteriasterol from star fish eggs (140), bombycesterol from silk worms and several others have not been proven to be chemical individuals, but the sterol of the oyster and other bivalves has recently been investigated (12). This ostreasterol melting at 140°C. is an isomer of stigmasterol, $C_{29}H_{47}OH$; hydrogenation of its two double bonds leads to sitostanol. Wool fat (lanolin) contains two sterols, lanosterol, $C_{30}H_{49}OH$, m.p. 141°C. and agnosterol, $C_{30}H_{47}OH$, m.p. 162°C.; they are not precipitable with digitonin, which increases the difficulty in the study of these interesting cutaneous excretion products (266).

H. Transitions. Modifications of the side chain

The structural identity or near-identity of sterols and bile acids was first verified experimentally by Windaus and Neukirchen (252), when they succeeded in the conversion of cholestane, by oxidation of the terminal group, into "allocholanic acid," and the conversion of coprostane ("pseudocholestane") into regular cholanic acid. Similarly, 3-chlorocholestane



CXXXIX
Norcholanyldiisopropylcarbinol (see LXXIII), $C_{30}H_{54}O$

CXL
Coprostanone-24, $C_{27}H_{46}O$

XXIX
Coprostane (see p. 321)

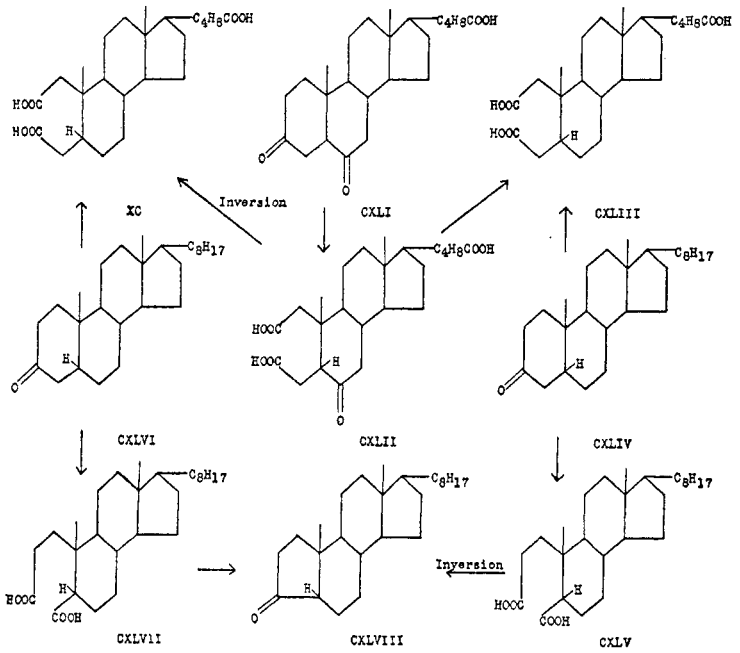
was converted into allolithocholic acid (260). Later, Wieland and Jacobi (238), reversed this process. Ethyl cholanate was converted by Grignard's reagent into the diisopropylcarbinol (CXXXIX) (cf. p. 331), which, in turn, on oxidation with chromium trioxide gave the isopropyl norcholanyl ketone (cholanyl-β-propane). This substance is nothing else than coprostanone-24 (CXL), and may be reduced to coprostane (XXIX) by zinc amalgam.

These interconversions between the sterol group and the bile acids have

been supplemented by a number of subsequent investigations. In some cases, inversion of the substituents on C_5 allows the preparation of both isomers from a common starting material, e.g., cholestene can be converted via its hydrochloride into coprostene, and while cholestene itself is hydrogenated to cholestane, coprostene is hydrogenated either to coprostane (= pseudocholestane) or to cholestane, depending on the procedure (see p. 320). The converse, where an identical product is attained from stereomeric sources, will be exemplified by three instances. (a) Hyodeoxycholic acid, itself a cholanic acid derivative, is dehydrogenated to a labile α-dehydrohyodeoxycholic acid, m.p. 162°C., $[\alpha]_D = -66°$, which suffers a Walden inversion on C_5 owing to the vicinity of the carbonyl group. (Intermediary enolization?) Oxidative cleavage of ring I in the stable β-dehydro acid (CXLI) of m.p. 209–210°C., $[\alpha]_D = 0$ (?), leads to 6-keto-isolithobilianic acid (CXLII), which is reduced by Kishner and Wolff's method to a mixture of two isolithobilianic acids. One of them is identical with Staden's acid (CXLIII) obtained from dihydrocholesterol via cholestanone (CXLIV) (255), the other one with isolithobilianic acid (XC) obtained from dehydrolithocholic acid and also from coprostanol via coprostanone (CXLVI). (b) Cholestanone, a saturated ketone, is oxidized to $C_{27}H_{46}O_4$, the dihydro derivative of Diels' acid (CXLV) (see p. 316). Coprostanone can be oxidized to a stereomeric dicarboxylic acid (CXLVII). Both acids yield the same pyroketone (CXLVIII) by thermic cyclization, which indicates, according to Windaus, that the center of asymmetry responsible for the isomerism of the acids must be vicinal to one of the carboxyl groups, i.e., in position 5.

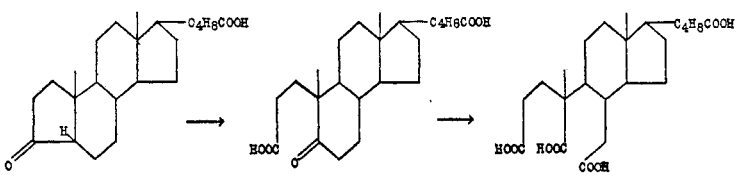
The reaction runs more smoothly and with better yield in the case of the "copro" isomer. As such cyclic pyroketones usually assume *cis*-configuration relative to the adjacent nucleus, this difference speaks for *cis*-configuration in the copro series, while the dihydro-Diels acid from cholesterol had to undergo an inversion of the carboxyl on C_5 before formation of the *cis*-ketone. (c) Wieland (231) carried out the following series of reactions: pyrolithobilianic acid (CXLIX) was oxidized by permanganate to 5-keto-3,24-dicarboxylic acid (CL), this in turn by hypobromite to the 3,5,6,24-tetracarboxylic acid (CLI), $C_{23}H_{36}O_8$. Its pyro derivative (CLII), $C_{22}H_{34}O_5$, is oxidized by nitric acid to the 3,5,7,24-tetracarboxylic acid (CLIII), $C_{22}H_{34}O_8$. This acid, m.p. 194°C., was identical with a tetrabasic acid obtained by Windaus (247) in the cholesterol series from Diels' acid (formula VIII). The last instance demonstrates the convergency of the two stereomeric series into a common end product through disappearance of the asymmetry of carbon atom C_5.

These synthetic links between the sterol series and the C_{24}-acid series series have found parallels in nature. Wieland and Kishi (234), were able



XC. Isolithobilianic acid (see p. 335)
CXLI. β-Dehydrohyodeoxycholic acid, $C_{24}H_{36}O_4$
CXLII. 6-Ketoalloisolithobilianic acid (= hyodeoxyisobilianic acid), $C_{24}H_{36}O_7$
CXLIII. Allo isomer of XC (Staden's acid), $C_{24}H_{38}O_6$
CXLIV. Cholestanone, $C_{27}H_{46}O$
CXLV. Dihydro-Diels' acid, $C_{27}H_{46}O_4$
CXLVI. Coprostanone, $C_{27}H_{46}O$
CXLVII. Copro isomer of dihydro-Diels' acid, $C_{27}H_{46}O_4$
CXLVIII. Dihydropyro-Diels' ketone, $C_{26}H_{44}O$

to isolate from the mother liquors of 10 tons of ox bile a few grams of a new acid, which is distinguished from all other bile acids by the number of carbon atoms. $C_{28}H_{46}O_4$ was given as a preliminary formula of this "sterocholic acid," a name suggesting a closer relation to the sterols. Other products which might fill the place of the missing link between sterols



CXLIX
Pyrolithobilianic acid, $C_{23}H_{36}O_3$

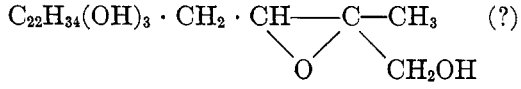
CL
Ketodicarboxylic acid, $C_{23}H_{36}O_5$

CLI
Tetracarboxylic acid, $C_{23}H_{36}O_8$

CLIII
Tetracarboxylic acid,
$C_{22}H_{34}O_8$

CLII
Pyro derivative of CLI,
$C_{22}H_{34}O_5$

and bile acids are tetrahydroxybufostane, $C_{27}H_{44}(OH)_4$ (?) found in toad bile (125), and especially scymnol. Although all vertebrate animals produce in their liver a biliary fluid, no bile acid in the proper sense is found in the bile of one of the phylogenetically lowest vertebrates, the shark (*scymnus borealis*). Scymnolsulfuric acid, discovered by Hammarsten (87a) in shark bile, is a sulfuric ester of scymnol, $C_{27}H_{46}O_3$, m.p. 187°C. Three of its oxygen atoms are apportioned to three secondary alcohol groups on C_7, C_{12} and in ring I, and the fourth one to a primary alcohol group on C_{27}, whilst the fifth is an ethylene oxide oxygen, forming a three-atom ring with C_{24} and C_{25} (184, 237, 240, 241, 265):



Certain toad poisons, studied by Wieland, by several Japanese investigators (109), and especially by K. K. Chen, H. Jensen, and A. L. Chen (271), are structurally related to the bile acids (see p. 313). No complete structural formulas have been established for them, owing to the scarcity of the starting material.

Rosenheim and Starling (160) recently obtained the elusive "oxycholesterol" ($C_{26}H_{46}O_2$, m.p. 236°C.) of Lifschütz (121) in the crystalline state by oxidation of cholesterol in various ways. An isomer melting at 176°C. was obtained (32) by the action of selenium dioxide on cholesterol. Both glycols, probably the *trans*- and *cis*-forms of 3,4-dihydroxycholestene ($\Delta^{5,6}$), rearrange easily into *copro*stenone, 3-ketocoprostene ($\Delta^{4,5}$). This unsaturated ketone yields on reduction coprostenol (allocholesterol). The latter is (a) easily reconverted into cholesterol, which hinders its detection as an intermediary in biological processes; (b) it can, in turn, be hydrogenated to coprostanol (coprosterol) which has never been obtained in vitro by direct reduction of cholesterol, but seems to be an end product of sterol metabolism; (c) coprostenol possesses the "copro" configuration and may thus be the biological predecessor of the bile acids.

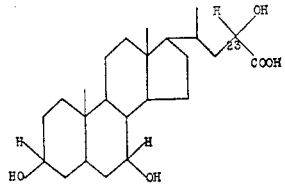
J. Degradation of side chain; hormones and carcinogenic products

Nothing is known in regard to the biological destruction of the bile acids. A bile acid with modified side chain is β-phocaecholic acid (CLIV), the 3,7,23-trihydroxycholanic acid referred to on p. 333. The typical hydroxyl group on C_{23} is located incidentally on one of the atoms which carry a double bond in the side chain of some of the sterols. Permanganate converts the bile acid of the seal into $C_{23}H_{38}O_4$, which is presumably 3,7-dihydroxynorcholanic acid.

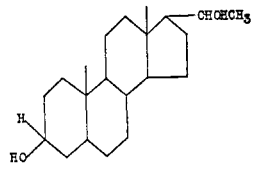
Pregnandiol (CLV), $C_{21}H_{36}O_2$, m.p. 236°C., a di-secondary alcohol recovered as a by-product in the isolation of female sex hormone from pregnancy urine is likewise a derivative of cholanic acid (30). One of its hydroxyl groups is most probably in position C_3 in accordance with all other natural products dealt with in this review; the other one belongs to the short side chain. The diol is oxidizable to a diketone, which in turn is reduced to the tetracyclic hydrocarbon pregnane (CLVI), $C_{21}H_{36}$, m.p. 83.5°C. This substance was also obtained by reduction of aetiocholanyl methyl ketone (CLVII), m.p. 115°C., prepared from bisnorcholanic acid methyl ester via the diphenylcarbinol and dehydration to the diphenylethylene (LXXVIII) (p. 332) and, finally, oxidation of the latter by ozone. While pregnandiol is physiologically inactive (71), a closely related unsaturated diketone $C_{21}H_{30}O_2$ (CLXIII), appearing in two polymorphous modifications melting at 120°C. and 128°C., has been recently identified as the active component of crystalline corpus luteum concentrates. (Isolation of crystals (272); structure (273); conversion of pregnandiol into "luteosterone" (274); preparation from stigmasterol (275).)

Removal of the last two carbon atoms (C_{21} and C_{20}) of the side chain leads to the male and female sex hormones. The male hormone, androsterone (CLXIV), m.p. 178°C., is a saturated hydroxyketone, $C_{19}H_{30}O_2$. Its structure, 3-hydroxyaetioallocholanone-17, has been made probable by Butenandt (276), and corroborated by Ruzicka (277), who synthesized it by oxidation from epidihydrocholesterol (ε-cholestanol) (cf. p. 346).

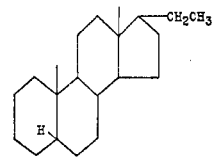
The follicular hormone $C_{18}H_{22}O_2$ (ketohydroxyoestrin = theelin) and its hydrate $C_{18}H_{24}O_3$ (trihydroxyoestrin = theelol) have been given the formulas CLVIII and CLIX. The older literature on isolation and properties of these substances was summarized recently by Marrian (128) and by Girard (75). For nomenclature see reference 3. These substances are the 3-hydroxy-17-keto and the 3,16,17-trihydroxy derivatives (126) of the hypothetical hydrocarbon oestratriene (CLX), $C_{18}H_{24}$, which is cholane minus the side chain C_{20} to C_{24} and the methyl group C_{10} and with three aromatic double bonds in ring I. Marrian and Haslewood (127) and McCorquodale, Thayer, and Doisy (123) opened ring IV of the trihydroxy hormone by alkali fusion.



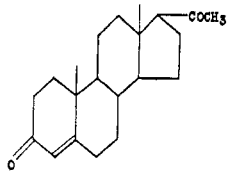
CLIV
β-Phocaecholic acid,
$C_{24}H_{40}O_5$



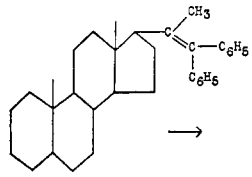
CLV
Pregnandiol,
$C_{21}H_{36}O_2$



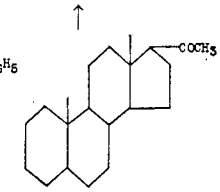
CLVI
Pregnane,
$C_{21}H_{36}$



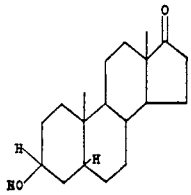
CLXIII
Corpus luteum
hormone,
$C_{21}H_{30}O_2$



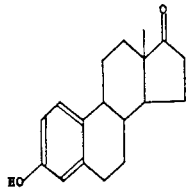
LXXVIII
Diphenylbisnorcholene,
(see p. 332)



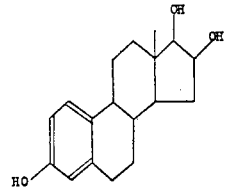
CLVII
Aetiocholanyl
methyl ketone
(=pregnanone-
20), $C_{21}H_{34}O$



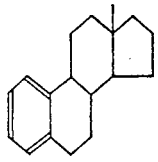
CLXIV
Androsterone,
$C_{19}H_{30}O_2$



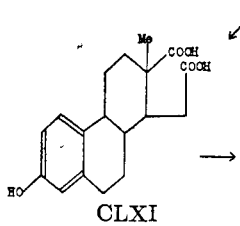
CLVIII
Ketohydroxyoestrin,
$C_{18}H_{32}O_2$



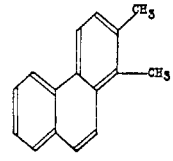
CLIX
Trihydroxyoestrin,
$C_{18}H_{24}O_3$



CLX
Oestratriene,
$C_{18}H_{24}$



CLXI
Phenolic dicarboxylic
acid, $C_{18}H_{22}O_5$



CLXII
Dimethylphenanthrene,
$C_{14}H_{14}$

The resultant phenolic dicarboxylic acid (CLXI) possesses the same skeleton as aetiobilianic acid (XIV). Complete dehydrogenation and simultaneous decarboxylation lead, as with aetiobilianic acid, to dimethylphenanthrol and subsequent distillation with zinc dust to the 1,2-dimethylphenanthrene (CLXII) mentioned on p. 334. The position of the hydroxyl group on C_3 (cholane nomenclature = position 7 in 1,2-di-

methylphenanthrene) is extremely probable, but awaits direct verification. This work establishes the close configurational and presumably genetic relationship between the sex hormones and the cholane and cholestane group.

The hydrocarbons obtained by "aromatization" suggest a connection between the compounds reviewed in these pages and the synthetic hydrocarbons of the London Cancer Hospital research group (38, 39, 40, 105, also 279). Their polycyclic aromatic hydrocarbons, usually embodying a phenanthroid constellation, display carcinogenic, and often also oestrogenic, activity, and are held responsible for the experimental generation of tumors by coal tar. Synthetic derivatives such as 1,2,5,6-dibenzanthracene belong here as well as methylcholanthrene (XXIVA) obtained from bile acid derivatives by aromatization. As "the cell proliferation which characterizes the oestrous state is in some respects reminiscent of the early stages of malignant growth" (quoted from reference 39), one may suspect that dehydrogenation of sterols and bile acids is the physiological method for the production of the sex hormone, but that further dehydrogenation by a faulty mechanism gives rise to carcinogenic substances (105, 128).

V. CHEMICAL AND PHYSICAL PROPERTIES OF BILE ACIDS

A list of all bile acid and sterol derivatives known to date would run into a total sum of five hundred and more, even if all functional derivatives such as esters, oximes, lactones, and halogen substitution products were disregarded. The biochemistry of the degradation products of proteins, carbohydrates, and lipoids has been intensely studied, since the products obtained by hydrolytic decomposition of these big molecules are identical with the intermediaries in animal and plant metabolism, e.g., amino acids, purine bases, monosaccharides, fatty acids, and so on. On the other hand, the numerous derivatives of bile acids (and sterols) have been all but ignored by biochemists; these polyannular molecules are considered laboratory products and they play no part in the biosynthesis of bile acids and sterols. The latter are synthesized by processes, still obscure, from simple molecules whose nature is yet a matter of conjecture; but these syntheses presumably follow lines quite different from those encountered in the structural analysis. This explains why the knowledge of the physicochemical and biochemical properties of this group of derivatives is almost entirely limited to its natural representatives, the conjugated bile acids and the free bile acids.

A. Analytical methods; color reactions

Some of the most characteristic chemical and physical properties of bile acids are involved in the methods developed for their analysis (43).

The oldest reaction for bile acids is the Pettenkofer reaction (142).

Bile acids, when heated with sugar in concentrated sulfuric acid, develop a beautiful purplish coloration. This reaction is by no means specific and even the numerous applications of modern colorimetric and spectroscopic methods, the latter also applied to the fluorescence of the Pettenkofer pigment, have never led to an accepted standard method (5, 34). The reaction was improved by Udransky (186), who supplanted saccharose by furfural, by Jolles (103), who recommended rhamnose or methylfurfural, and by Inouye and Ito (100), who used vanillin. The presence of other potential contributors to a positive Pettenkofer test, as terpenes etc., has been excluded by chemical isolation of the bile acids as salts, for instance as quinine salt (191), prior to the performance of the color test. In some instances, the Pettenkofer reaction has resulted in interesting biological findings, but its quantitative evaluation has never escaped controversial ground.

Enderlen (62) observed a brilliant blue color reaction when replacing the concentrated sulfuric acid by dilute, yet strong, sulfuric acid. This reaction has been rediscovered by Gregory and Pascoe (80), and studied by Reinhold and Wilson (152) and by Doubilet (58). It is specific for cholic acid and its conjugated derivatives; deoxycholic acid and other dihydroxycholanic acids and their conjugation products fail to produce the blue substance with furfural and 70 per cent sulfuric acid except for faint traces which might still be due to contamination with cholic acid. Whereas this method promises to permit differentiation among the various bile acids, one has to turn to some other means for the determination of total bile acids. A general method, harboring this promise, has been proposed by Szillard (181); the insolubility of the ferric salts of bile acids and a quantitative determination of the iron by one or another titrimetric or colorimetric reaction forms a sound stoichiometric basis for quantitative estimation.

Liebermann's color reaction with acetic anhydride and concentrated sulfuric acid, used widely in the analysis of cholesterol, is also given by a number of bile acid derivatives, but can not be used for their estimation. (229, p. 277).

A very valuable aid in the analysis of conjugated bile acids is the determination of amino nitrogen by van Slyke's method, as introduced by Foster and Hooper (67) and Schmidt and Dart (168). Its results have to be interpreted with discrimination, since the possibility of the presence of nonconjugated bile acids in physiological and pathological specimens can not be dismissed (77, 169).

B. Physical properties

An analytical method based on physical properties of the bile acids is the polarimetric determination of purified extracts (102). The value of

this procedure is impaired by the differences in specific rotation between the bile acids occurring in varying proportion in biological fluids where quantitative estimation is needed.

The high viscosity of blood serum in jaundice is due to the presence of bile acids, but the quantitative relations of this property are not sufficiently clarified as to permit interpretation in terms of bile acid content.

The immense surface activity of bile acids has been utilized for a long time in the so-called Hay test (92, 72). Flowers of sulfur is strewn on the surface of a fluid suspected to contain bile acids. The specific gravity and the size of the particles of the sulfur are such that they will float on water (s = 73 dynes per square centimeter) and on liquids with a moderately reduced surface tension as long as the latter is above 52 to 56 dynes per square centimeter. If the surface forces drop below this value, they cannot hold the sulfur particles which, in consequence, sink to the bottom. The positive outcome of this test will establish a minimum content, in terms of one of the bile acids chosen as a standard, and subject to the nature and amount of other constituents of the sample, especially proteins. Quantitative surface tension measurements can be made with a variety of procedures such as capillary methods, stalagmometric methods, and especially by estimation of the static surface tension, using a ring, blade or stirrup (61, 78) in connection with a mechanical or electrical torsion balance. Influence of interfering substances, such as polyvalent electrolytes (phosphates) or colloids (proteins), can be eliminated by operation in an acid medium, and by comparison with a blank measured after absorption of the bile acid on charcoal (60). Comparisons of the surface activity of free and unconjugated bile acids will shed further light on the analytical possibilities of this property (59, 63).

C. Monomolecular layers

The progress of our knowledge of monomolecular layers will eventually allow an interpretation of the data on surface forces in the light of molecular dimensions. Studies on monomolecular layers have already been undertaken by Adam, Rosenheim, and Danielli (1, 2, 51, 52) in the sterol series, and their results regarding the cross section of the sterol molecule are in perfect agreement with x-ray data. Views regarding the tilt of the molecules in such layers, the polarity of the functional groups, the position of double bonds, as revealed by their behavior when spread on permanganate solutions, are additional achievements of these studies.

D. X-ray analysis

As mentioned in the introduction, x-ray spectroscopy and optical crystallography "pointed clearly to the fact that the older accepted formulas

(sc. for the sterols) could not be made to fit into the crystallographic cell" (13). In the case of ergosterol, for instance, the formula proposed by Wieland and Windaus requires cell dimensions of 8.5 × 7.0 × 18.0 A.U. or according to Wieland's modified formula (228) 11.0 × 7.5 × 15.0 A.U. Rosenheim and King's formula fits into a cell of 7.5 × 4.5 × 20.0 A.U., while Bernal observed 7.2 × 5.0 × 20.0 A.U. for ergosterol. Similar confirmations of Rosenheim and King's theory are obtained from the molecular dimensions of pregnandiol and ketohydroxyoestrin. Cholic acid does not give such clear evidence, as the packing is more complex owing to the various functional groups. However further study, especially of the underlying hydrocarbons, promises a general elucidation of these points and a correlation with other phenanthroid nuclei as in Jacobs' hydrocarbon $C_{16}H_{14}$ (101) from strophanthidin (Bernal in reference 52). For x-ray measurements of choleic acids see p. 360.

The results of ultra-violet spectroscopy have been consulted with great advantage in the study of unsaturated sterol compounds, especially ergosterol and its irradiation products (T. M. Lowry in reference 52). An investigation by Menschick, Page, and Bossert (133) had a more direct bearing on the chemistry of bile acids. They concluded from the ultraviolet absorption curve of coprostenone (old nomenclature: "cholestenone") (XV), that it belongs to the type of α,β-unsaturated and not to that of β,γ-unsaturated ketones. This fact could not easily be reconciled with the old sterol formula, but its implications regarding oxidative destruction of coprostenone dovetail with all other evidence in favor of the new formula.

The effects of irradiation on ergosterol are beyond the scope of this review. Its effects on the steric configuration of cholic acid described by Uraki (187) require further confirmation.

E. Acid dissociation constants

Qualitative observations on the relative acidity of bile acids have been utilized in the separation of lithocholic acid from the more acid deoxycholic acid (see p. 333). Phocaecholic acid, an α-hydroxy acid, would be expected to be among the strongest acids in this group.

Quantitative studies on the acid dissociation of bile acids are scarce. Bondi, from conductivity measurements (19), estimated the dissociation constant of glycocholic acid at $k = 1.32 \times 10^{-4}$ (pK = 3.87). He gives 1.38×10^{-4}, 0.18×10^{-4}, and 2.2×10^{-4} for the dissociation constants of lactic, acetic, and hippuric acids, respectively. From the ratio of the constants for hippuric acid and benzoic acid (2.2×10^{-4}: 0.6×10^{-4}), he deduces that the dissociation constant of free cholic acid must be less than 0.6×10^{-4} (pK = 4.22). Henriques (95) used the method of Michaelis and Mizutani (134), which consists in the colorimetric comparison of an

unknown solution with a known buffer solution of glycine plus acetic acid in alcohol. He obtained pK = 2.78 and 3.23 for glycocholic and taurocholic acid (Bondi's $k = 1.32 \times 10^{-4}$ corresponds to a pK of 3.87) and 2.46 and 2.93 for "glycocholeic and taurocholeic acid," expressions that should be replaced by glycodeoxycholic and taurodeoxycholic acid. Josephson (104), working with electrometric methods on aqueous solutions of perhaps purer materials, obtained the following values for pK (those marked with an asterisk corrected for activity coefficients): cholic acid, 5.19–5.20*; deoxycholic acid, 6.42–6.45*; glycocholic acid, 4.45–4.51*; glycodeoxycholic acid, 3.81; taurocholic acid, 1.39; taurodeoxycholic acid, 1.74. The low values (strong acidity) of the tauro acids are in good agreement with the older values of pK = 1.17 for taurocholic acid (4.40 for glycocholic acid), given by H. Hammarsten (86). These important constants deserve further study, e.g., by the method of Kuhn and Wassermann (110) for the determination of dissociation constants of acids scantily soluble in water.

VI. CHOLEIC ACIDS

We have reserved until here the discussion of one group of bile acids, the choleic acids. The term "Choleinsaeure," coined by Demarçay (45), had covered an ever-changing complex of bile educts (183) until it was fixed by Lachinov (114) upon a bile acid which accompanied cholic acid in varying proportion. This deconjugated, i.e., non-nitrogenous, bile acid showed certain similarities with Mylius' deoxycholic acid (135) and could be converted into deoxycholic acid containing "crystal-acetic acid" by treatment with glacial acetic acid. The question as to the identity or diversity of these two substances remained undecided, while the terms choleic and deoxycholic acid were used indiscriminately, e.g., by Wahlgren (194) and Gullbring (84), who isolated glycocholeic acid (= glycodeoxycholic acid) and taurocholeic acid (= taurodeoxycholic acid) in Hammarsten's laboratory.

All incongruities were clarified at once through the ingenious conception of the "choleic acid principle" by Wieland and Sorge (200). In an attempt to study the effect of high vacuum distillation on choleic acid, a method which had proven useful in the case of cholic acid, these authors observed the appearance of palmitic, stearic, and oleic acids in the head fraction of the distillate to an extent of about 8 per cent of the choleic acid employed. The remainder proved identical with deoxycholic acid. Prompted by Werner's achievements in complex compound chemistry, they realized that they were dealing with a complex or coördination compound consisting of eight molecules of deoxycholic acid plus one molecule of fatty acid. This molecular compound had a higher melting point (186°C.) than either

of its constituents, deoxycholic acid melting at 172°C. It differed from it in its solubilities, but since the fatty acid could be replaced by an excess of acetic acid, acetone, alcohol, or ether, it can easily be converted into deoxycholic acid, which is known to crystallize with one molecule of one of the solvents mentioned for each molecule of bile acid. The complex "choleic acid" can be resynthesized from fatty acid and deoxycholic acid.

Further experiments showed that other fatty acids, as butyric acid, and also aromatic substances, like phenol and naphthalene, were able to enter coördination compounds with deoxycholic acid in varying but characteristic molecular proportions. The term "choleic acid" was now widened to comprise these addition compounds, and the choleic acid containing stearic, palmitic, or oleic acid, and isolated from animal bile, was designated "natural choleic acid."

To the formation of these compounds was ascribed the important physiological function of bile acids to render all sorts of water-insoluble substances water-soluble. Since the alkali salt, e.g., of naphthalenecholeic acid or camphorcholeic acid, is water-soluble, naphthalene, camphor, etc., can thus be held in aqueous solution. The significance of this principle for the theory of intestinal resorption is obvious. While the physical process of emulsification can not be ignored, any explanation of lipoid resorption and transport would be incomplete without the choleic acid principle.

This peculiarity of deoxycholic acid is shared only to a limited extent by other bile acids. Cholic acid combines with various alcohols and mercaptans according to Mylius (135a); dehydrocholic acid crystallizes with benzene in the molecular proportion 2:1 (37). Various acetyl and halogen derivatives of hydroxy-, hydroxyketo-, and keto-cholanic acids crystallize with acetic acid, acetone, ethyl acetate, alcohol, ether, etc. But all these acids combine with the solvent in the molecular proportion 1:1; thus they do not distinguish themselves from many other known organic compounds.

The existence of unusual coördinative valence in this group is responsible for many double compounds such as "Weyland's acid," which was shown by Wieland and Kishi (234) to consist of one molecule each of anthropodeoxycholic (= chenodeoxycholic) acid and 3-hydroxy-12-ketocholanic acid. Likewise, in the sterol series, γ-cholestanol was recognized as a double compound of cholestanol with epicoprosterol (250). Page and Mueller (141) demonstrated the presence of a double compound of cholesterol and dihydrocholesterol in human brain. The combination of various sterols with digitonin and other saponins, discovered by Windaus (254), should be mentioned in this connection.

Returning to deoxycholic acid itself, one has to record that a great many careful observers before Wieland and Sorge had noted the stubborn tenacity by which fatty acids are retained whenever the purification of bile

acids had been attempted (e.g., 112, 113). This was perhaps due to the presence of deoxycholic acid, which in turn is not easily separated from cholic acid.

Boedecker and Volk (17) discovered that this property of deoxycholic acid was duplicated in the behavior of apocholic acid (but not of the isomeric dihydroxycholenic acid). This fact hints at the specific enhancing influence of the hydroxyl groups in positions C_3 and C_{12} on coördinative valence. If we compare deoxycholic acid and apocholic acid on one side, with cholic acid, lithocholic acid, and cholanic acid on the other side, all of which have no pronounced coördinative power, we are led to the following assumption. The residual valences in the second group of acids are scattered in various directions from the main longitudinal axis of the molecule which stretches from the hydroxyl on C_3 in lithocholic acid to the distal common carboxyl group in position 24 at the end of the side chain. In deoxycholic and apocholic acid, the second hydroxyl (C_{12}) seems to direct these residual affinities to the opposite front of the molecule and we incline to the view that the unbroken chain of aliphatic carbons C_3 C_4 C_5 C_6 C_7 C_8 C_{14} C_{15} C_{16} C_{17} C_{20} C_{22} C_{23} C_{24} is endowed with this particular clinging power. (This chain would be interrupted by a double bond between C_7 and C_8 in the dihydroxycholenic acid isomeric with apocholic acid.) The question why cheno- or hyo-deoxycholic acids do not exhibit similar affinity on the other side of the molecule (chain C_3 C_2 C_1 C_{10} C_9 C_{11} C_{12} C_{13} C_{17} C_{20} C_{22} C_{23} C_{24}) finds its answer by inspection of the spatial model. This front of the molecule is interrupted by the methyl groups C_{18} on C_{10}, C_{19} on C_{13}, and C_{21} on C_{20}. This hypothesis might be tested in the future by methods of "molecular anatomy." A start in this direction was made by Herzog, Kratky, and Kuriyama (96), who studied the x-ray structure of stearic acid–choleic acid, with the result that the crystal cell was found only to occupy one-half of the minimum molecular volume as computed from molecular weight and density of a stearic acid–octocholeic acid. Perhaps the eight molecules of bile acid are arranged in two tetrades around the upper and lower half of the stearic acid chain, allowing two "identities" or pseudo-identities within one molecule of the coördination compound. Further studies on the crystal structure of choleic acids, carried out by Go and Kratky (76), indicate that crystals of choleic acids of coördination number 2 and 6 likewise contain cells, comprising four molecules of deoxycholic acid, thus corresponding to two molecules, and $\frac{2}{3}$ of a molecule, respectively, of the choleic acid.

Which substances function as partners in choleic acids? A great many compounds have been studied, especially by Rheinboldt and his coworkers (153) and by Sobotka, Goldberg, and Kahn (175, 176, 177), who sum-

marize them under the term of "acholic constituent" by analogy with the "aglucone" of glucosides.

A. Coördination number

While Wieland and Sorge originally assumed that the number of deoxycholic acid molecules combining with the monocarboxylic acids of the aliphatic series was in the simple proportion of one molecule of bile acid for each $—CH_2—CH_2—$, systematic research by Rheinboldt showed that only those coördination numbers can be verified in choleic acids which have been recognized as the general architectural principle in inorganic coördination compounds since Werner. These numbers must allow for a symmetrical arrangement around the pivotal atom, ion, or group. The numbers commonly encountered are 4, 6, 8, occasionally 2, and 3, while the numbers 5 and 7 would not permit symmetrical constellation. Potential higher coördination numbers are 12 and 20, according to Hüttig (98), but no evidence has been adduced for the existence of such huge symmetrical molecular aggregates. Figure 1 gives the coördination number of homologous aliphatic monocarboxylic and dicarboxylic acids. Physical properties in homologous series, such as melting points, solubility, acid dissociation constant, surface activity, etc., change regularly and gradually with the molecular size, i.e., with the number of carbon atoms, or follow an oscillatory course between the homologs with an even and those with an odd number of carbon atoms. In contradistinction, coördinative valence in an homologous series is not a gradual, but a periodical function of molecular size and to such a factor one might ascribe peculiarities and specificities in the biochemical behavior of homologous members.

Rheinboldt found that an alcohol with n carbon atoms has the same coördination number as the acid with $(n + 1)$ carbon atoms. In alkyl esters of aliphatic acids, the coördination number peculiar to the longer of the two chains prevails. Halogenated aliphatic acids (and in some instances unsaturated acids) behave as a rule like the saturated acids of equal carbon number. The influence of branched chains upon coördinative valency was studied by Sobotka and Goldberg, who found the coördination number reduced to that of the longest straight chain contained in the molecule. The coördination number 3 was observed in crotonic acid, in some enol derivatives, and in aromatic substances such as phenol. Whether this is a peculiarity of unsaturated and aromatic compounds must be decided by a survey of a greater number of choleic acids. Chargaff and Abel (278) likewise found decreased coördination numbers for α-alkylated fatty acids; they obtained no choleic acids at all with halogenated fatty acids.

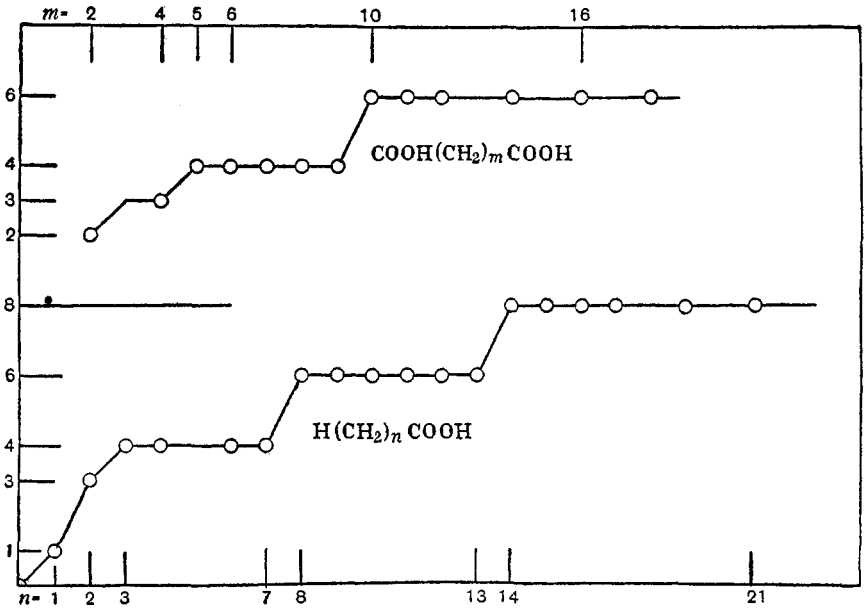


FIG. 1. RELATION BETWEEN LENGTH OF ALIPHATIC CHAIN AND COÖRDINATION NUMBER

Sobotka and Goldberg (175)

B. Coördination compounds and optical activity

While the determination of coördinative valence towards bile acids offers a quantitative approach to several problems, there are additional features of even greater significance to be taken into account. Choleic acids vary not only as to coördination number but also as to their stability. This is a function of volatility and solubility of the acholic component, and of the degree of "saturation" of the coördinative valence. One acholic constituent might dislodge another owing to its greater coördinative affinity, a reaction that has been utilized by Wieland and by Rheinboldt in the analysis of aliphatic acid–choleic acids which dissociate in xylene to insoluble xylenecholeic acid and free aliphatic acid. Differences in physical properties enabled Sobotka and Goldberg (176) to isolate choleic acids of levorotatory camphor, phenylethylethanol, dipentene, and methylethylacetic acid from solutions of the respective racemic acholic components and deoxycholic acid. Owing to the optical activity of the bile acid, *l*-camphorcholeic acid and *d*-camphorcholeic acid are not mirror images of each other, but differ in their physical properties and can thus be separated by crystallization. This phenomenon offers a possibility of resolving racemic hydrocarbons and other substances, devoid of functional groups that would allow resolution by the customary Pasteurian methods.

C. Multiple coördination numbers

These have been encountered in several acholic substances; in other words, some compounds form more than one choleic acid. Rheinboldt observed the existence of camphormonocholeic and camphordicholeic acids (154). Depending on the method of synthesis, two choleic acids each were obtained by Sobotka and Goldberg from butyric acid and from its ethyl ester. The butyric acid–choleic acid with coördination number 2 was prepared by direct synthesis, i.e., by dissolution of solvent-free deoxycholic acid in butyric acid. If, on the other hand, butyric acid and deoxycholic acid are combined in alcoholic solution, without an excess of butyric acid, then four molecules of deoxycholic acid will combine with a single molecule of butyric acid. Thus, the initial concentration of the two compounds influences the coördinative ratio.

The presence of alcohol or some such solvent seems to compete with the acholic constituent for the deoxycholic acid; therefore, many substances can not be induced to form choleic acids in alcoholic solution, e.g., glutaric acid and various branched-chain aliphatic acids. The direct method of synthesis is indispensable in these cases. In instances of multiple coordinative valence, the direct method will yield the compound with lower coördination number.

A third method of synthesis consists in the addition of the barely water-soluble acholic component, e.g., ethyl butyrate, to an aqueous solution of sodium deoxycholate. The lipoid phase solidifies gradually and the resultant crystalline layer is ethyl butyrate–dicholeic acid. This last method also illustrates how the addition of a non-electrolyte phase to a bile salt solution influences the ionic concentrations of the aqueous phase through hydrolytic absorption. The sodium ion is left in the aqueous phase and the pH of this phase is raised.

D. Tautomerism and coördination

The study of ethyl acetoacetate choleic acid and the choleic acids of related tautomeric diketones yielded results of twofold interest (177). While ethyl acetoacetate in liquid form or in solution contains between 8 and 13 per cent of enol, it is 100 per cent enolized in its choleic acid. The corresponding figures for acetylacetone are 70 per cent enol in solution and 100 per cent in combination with deoxycholic acid. Through the crystallization of the choleic acid the tautomeric equilibrium is disturbed and the entire amount is gradually enolized. When the crystals of these choleic acids are dissolved, they undergo molecular dissociation and the liberated enol is ketonized at a measurable rate until the tautomeric equilibrium is established. Very quick operation however shows that maximal enol percentages approximating 100 per cent are reached only 30 seconds to a

minute after dissolution, which indicates that molecular dissociation proceeds with a high but measurable speed.

The assumption that choleic acids when dissolved are subject to complete or at least partial molecular dissociation is further supported by molecular weight determinations. Such determinations were carried out by Sobotka and Goldberg in Signer's apparatus (174), which utilizes the principle of isothermic distillation of Barger, on sebacic acid–choleic acid. The apparent molecular weight in methyl alcohol indicates practically complete dissociation into sebacic acid and eight molecules of deoxycholic acid.

Both experiments, the titration of free enol in the case of acetoacetic ethyl ester, and the molecular weight determination in the preceding example, were performed in alcoholic solution, thus in a solvent which has an affinity of its own towards bile acid. Different conditions may exist in aqueous solution, where interrelation of molecular dissociation and electrolytic dissociation of the bile acid and also of the acholic component, if an acid, have to be considered (cf. p. 363, synthesis from aqueous solution). This subject is of great importance for the application of Wieland's choleic acid principle to alimentary adsorption.

E. Resorption in the intestinal tract

One has to keep in mind that animal bile contains conjugated bile acids. No choleic acids with conjugated bile acids have been unequivocally isolated (170), but the conjugated acids share the ability of the free bile acids of keeping water-insoluble substances in solution. The capacity of free bile acids to keep fatty acids and other acholic components in aqueous solution, whether due to actual coördination compounds or to surface forces, is confined to the alkaline side of the neutral point (189). Conjugated bile acids, owing to their greater acidity, retain this dissolving power even in slightly acid reaction down to pH 6.

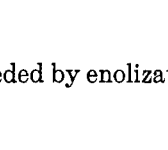
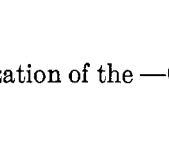
Another angle from which this problem can be approached is the study of diffusion in mixtures containing bile acids (188, 73). The present state of knowledge is still limited, as the influence of pH, the nature of the membrane, and several other factors have to be considered individually. One may surmise that combination with bile acids will increase the diffusibility of substances less diffusible than the bile acids themselves, while it will cause the retention of substances more diffusible. Furthermore, the influence of the bile acids will be enhanced towards more alkaline solutions. These conditions may be complicated in the animal body (a) by enzymatic reactions disturbing the equilibrium on either side of the membrane, e.g., hypothetical resynthesis of neutral fat within the duodenal villi, (b) by the peculiar physical properties of such substances as lecithin, which exert an influence of their own on solubility in mixtures and may supplement the

effect of the bile acids (73). The observation that bile acids may promote the diffusion of multiple amounts of lipoids through the intestinal mucosa may be explained thus: the bile acid complex is absorbed on the membrane, and the acholic component is removed by diffusion, while the bile acid returns into the solution (189).

The resorption of drugs, vitamins, and hormones is also linked up with the circulation of the bile. Wieland suggested that the resorption of alkaloids such as strychnine in the alkaline intestinal tract is based on the choleic acid principle. (Cf. e.g., Kolda (107) for alkaloids; Mendel and Daniels (132) and Stockholm and Schmidt (180) for fat-soluble dyes; Crowe (42) for hexamethylenetetramine). This idea has been utilized in the simultaneous administration of synthalin with sodium dehydrocholate. The failure of the peroral administration of insulin–bile salt mixtures to produce hypoglycemia (179, 74) is not surprising when one realizes the chemical nature of this hormone. On the other hand, the female sex hormone, owing to its lipoid character, has a definite affinity towards bile: it has been detected in the gall bladder of an old man (70, 81) and its ability to form a choleic acid was put to preparative use by Wieland, Straub, and Dorfmueller (239). The relationship between vitamin A and bile acids postulated by Shimizu and Hatakeyama (172) must be examined in the light of the modern knowledge of this vitamin. Seyderhelm (171) demonstrated the necessity of peroral administration of bile acids for the resorption of the fat-soluble vitamin D to counteract the typical anemia developing in dogs whose bile flow is diverted from the intestine through a bile fistula.

The enolizing influence of bile acids, mentioned above, has been adduced to explain the difference between the direct and indirect diazo reaction of bilirubin according to Hymans van den Bergh. Bilirubin in the serum of jaundiced subjects seems to occur in two forms. One form, suspected to be the enol, couples readily with diazotized sulfanilic acid, while the other one reacts only after it has been extracted with alcohol. A study of the incidental pathological conditions suggests that the simultaneous presence of bile acids is prerequisite for the "direct" reaction, while the less frequent sera with "indirect" reaction only contain the bilirubin in the keto form and require the alcohol treatment for enolization (69, 177). The enolizing influence of bile acids may play a part in the beta-oxidation of fatty acids.

The influence of the bile acids on enzymatic reactions is especially noticeable with the lipolytic enzymes. Lipase, the enzyme splitting triglycerides, is enhanced in its action by bile and bile acids, while esterase, the intracellular enzyme of the liver, which is most active towards simple aliphatic esters, is inhibited by bile salts. This antagonism may find its explanation in the surface activity of the bile acids, but since hydrolysis might be

preceded by enolization of the  group to  , we

can conceive of more specific reasons for these phenomena.

VII. CONCENTRATION AND pH OF BILE

Bile as excreted from the liver displays an alkaline reaction, varying from 7.8 to 8.6 in various species (see references 137, 139, 146). It contains on the average 1 to 4 per cent of solid constituents; these are bile acids in the form of their sodium salts (in some marine animals also a high amount of potassium salt), minor amounts of lecithin and cholesterol, inorganic constituents of the same type as in blood serum, with sodium chloride prevailing, and fluctuating amounts of mucin, in part contributed by the lining of the bile ducts. This liver bile is stored in many species in the gall bladder, where it undergoes concentration at a remarkable rate (162). Gall-bladder bile might contain as much as 20 per cent of total solids as a result of a tenfold concentration within a few hours. It seems paradoxical, then, that both liver bile and gall-bladder bile should be isotonic with blood serum and the other body fluids. But this fact has been ascertained by Brand (27) through freezing-point determinations. Brand revealed that the concentration of the inorganic constituents is gradually reduced through diffusion from 0.8 per cent to 0.2 per cent, while the organic components increase. These conditions must be governed by Donnan's law, as we are dealing with a mixture of sodium chloride and sodium bile salts within the bladder and a solution of sodium chloride in the outside fluid, the blood of the capillary system of the bladder wall that takes up the fluid. Although the constant flow of the blood and of the bile prevents the establishing of a true equilibrium, the bladder bile turns more acid and, in concentrated state, reaches pH values below 6 (151). The influence of this shift of sodium and hydrogen ions upon the capacity of the bile to keep lipoids, especially cholesterol, in the dissolved state and upon its diffusibility is not yet understood. We know only that those human gall bladders in which stones are found at autopsy regularly show a ratio bile acids: cholesterol of less than 8:1 by weight, while stone-free bile contains a higher relative amount of bile acid, regardless of the absolute amount of solid ingredients in either group (136).

VIII. PHYSIOLOGICAL AND PHARMACOLOGICAL OBSERVATIONS

Bile participates in the detoxifying function of the liver, in the case of both organic and inorganic poisons. There are numerous references to the

presence of heavy metals in the bile in poisoning (lead (7), manganese (148), cresol as glucuronic derivative (16)). The affinity of the bile for foreign organic substances is best exemplified by the accumulation of tetraiodophenolphthalein and other radiopaque chemicals, used in the visualization of this organ, and of organic mercury compounds and other disinfectants which were introduced into the body with the aim of sterilizing the infected gall bladder (typhoid carriers, etc. (138)).

The physiological and pharmacological literature abounds with studies on bile fistulas. There is hardly a group of substances that has not been tried for its cholagogue, choleretic, or chologenic value. Choleretic action designates the stimulation of the liver to excrete more bile, while cholagogue action refers to compounds which stimulate the emptying of the gall bladder. Of greater interest to the chemist is the study of chologenic substances, namely, substances that can be identified as actual starting material from which bile acids are synthesized. Such synthesis has not been demonstrated up to the present time, except for the observation that the output of taurocholic acid depends on the supply of cystine sulfur (11, 68). Despite the absence of conclusive physiological evidence, all that has been disclosed in regard to the constitution of sterols and bile acids, especially the new data on "oxycholesterol" (160), will induce the chemist to suspect that bile acids are formed in the liver by oxidative processes from a compound closely related to cholesterol.

Bile under physiological conditions is confined to the well-known cycle liver–gall bladder–duodenum–lymph spaces–portal system–liver. Under pathological conditions, the bile acids as well as other bile constituents, will trespass into blood serum and urine. The presence of bile acid in urine has been demonstrated by many methods. Its occurrence in the blood is inferred by its appearance in the kidney excretion, but its direct quantitative estimation in the blood depends on the establishment of more exact analytical methods (see pp. 354 to 356).

The toxic action of bile acids on the animal organism has been studied by the pharmacologist Hermann Wieland (242) by intravenous injection into animals or surviving preparations of heart and muscles. Especially does its effect on the heart resemble that of other surface-active substances like the saponins. Its lytic effect on single cells, as the red blood corpuscles or the pneumococcus, while of practical importance, is non-specific and quantitatively inferior to the lytic action of fatty acids. Deoxycholic acid produces greater effects than cholic acid in most of the biological phenomena enumerated, but we hesitate to ascribe this solely to its greater coördinative affinity. On the other hand, deoxycholic acid is more easily detoxified by the serum proteins than cholic acid, so that their final action, e.g., on the heart after intravenous injection, is about equal, according

to Hermann Wieland. The part played by the white blood corpuscles in the detoxification of bile acids has to be further investigated (41). The use of hemolysis and other biological phenomena in the analysis of bile acids, as suggested by some authors, can not be recommended.

The final fate of the bile acids in the animal organism is even more obscure than their origin. The tetracyclic carbon skeleton of the sterols, the bile acids, and the sex hormones has earned its wide-spread occurrence in nature by its great stability. It is hardly attacked by microörganisms and will persist in the carcass as adipocere long after carbohydrates, lipoids, and proteins have fallen a prey to the various saprophytes. No fragments that would offer a clue to the manner of its catabolism have been found in the living or dead animal body nor in its excretion products.

IX. CONCLUSION

We hope to have demonstrated in the foregoing pages (a) that we now possess a formula for the bile acids and related substances which agrees with the known chemical and physical facts and harmonizes with our general conceptions of the structure of natural substances, (b) that the possibility of syntheses in this group has been brought within our grasp, (c) that natural synthesis and catabolism of bile acids is still terra incognita, (d) that the establishment of the constitution opens the way for intelligent interpretation and research of the physicochemical properties of bile acids, and (e) that such knowledge will cause the numerous physiological, pharmacological, and pathological facts and observations, accumulated in the past, to be understood and correlated in the future.

REFERENCES

(1) ADAM, N. K., AND ROSENHEIM, O.: Proc. Roy. Soc. London **126A**, 25 (1929).
(2) ADAM, N. K., DANIELLI, J. F., HASLEWOOD, G. A. D., AND MARRIAN, G. F.: Biochem. J. **26**, 1233 (1933).
(3) ADAM, N. K., ET AL.: Nature **132**, 205 (1933). Nomenclature of the oestrin group.
(4) ADICKES, F.: Ber. **58**, 211 (1925).
(5) ALDRICH, M., AND BLEDSOE, M. S.: J. Biol. Chem. **77**, 519 (1928).
(6) ANDERSON, R. J.: J. Am. Chem. Soc. **46**, 1717 (1924); **48**, 2972 (1926).
(7) ANNUSCHAT, A.: Arch. exptl. Path. Pharmakol. **7**, 45 (1877).
(8) ASCHAN, O.: Ber. **45**, 1604 (1912).
(9) BAEYER, A. VON, AND VILLIGER, V.: Ber. **32**, 3625 (1899).
(10) BERGMANN, E., AND HILLEMANN, H.: Ber. **66**, 1302 (1933).
(11) BERGMANN, G. VON: Hofm. Beitr. **4**, 192 (1903).
(12) BERGMANN, W.: J. Biol. Chem. **104**, 317, 553 (1934).
(13) BERNAL, J. D.: Nature **129**, 277 (1932); Chemistry & Industry **51**, 466 (1932).
(14) BERNAL, J. D., AND CROWFOOT, D.: Chemistry & Industry **52**, 729 (1933).
(15) BLANC, H. G.: Compt. rend. **144**, 1356 (1907).
(16) BLUMENTHAL, F.: Biochem. Z. **1**, 135 (1906).
(17) BOEDECKER, F., AND VOLK, H.: Ber. **53**, 1852 (1920); **54**, 2302, 2489 (1921).

(18) BONDI, S., AND MÜLLER, E.: Z. physiol. Chem. **47**, 499 (1906).
(19) BONDI, S.: Z. physiol. Chem. **53**, 8 (1907).
(20) BONDZYNSKI, ST., AND HUMNICKY, V.: Z. physiol. Chem. **22**, 396 (1897).
(21) BORSCHE, W., AND ROSENKRANZ, E.: Ber. **52**, 342 (1919). Untersuchungen über der Konstitution der Gallensäuren. Paper No. 1.
(22) BORSCHE, W., AND FRANK, R.: Ber. **59**, 1748 (1926). Paper No. 9.
(23) BORSCHE, W., AND FRANK, R.: Ber. **60**, 723 (1927). Paper No. 10.
(24) BORSCHE, W., AND FRANK, R.: Ber. **60**, 1216 (1927). Paper No. 11.
(25) BORSCHE, W., AND FESKE, E.: Z. physiol. Chem. **176**, 109 (1928). Paper No. 13.
(26) BORSCHE, W., AND TODD, A. R.: Z. physiol. Chem. **197**, 173–90 (1931). Paper No. 15.
(27) BRAND, J.: Pflüger's Arch. **90**, 491 (1902).
(28) BRAUN, J. VON, AND IRMISCH, G.: Ber. **65**, 883 (1932).
(29) BREDT, J.: Ann. **437**, 1 (1923).
(30) BUTENANDT, A.: Ber. **63**, 659 (1930); **64**, 2529 (1931).
(31) BUTENANDT, A., WEIDLICH, H. A., AND THOMPSON, H.: Ber. **66**, 601 (1933).
(32) CALLOW, R. K., AND ROSENHEIM, O.: Chemistry & Industry **52**, 272 (1933); J. Chem. Soc. **1933**, 387.
(33) CERECEDO, L. R.: Thesis, Munich, 1921.
(34) CHARLET, M.: Biochem. Z. **210**, 42 (1929).
(35) CLEMMENSEN, E.: Ber. **46**, 1839 (1913); **47**, 51, 681 (1914).
(36) CLEVE, P. T.: Bull. soc. chim. **35**, 373, 429 (1881); **38**, 131 (1882).
(37) COHN, LASSAR: Ber. **25**, 803 (1892).
(38) COOK, J. W., DODDS, E. C., AND HEWETT, C. L.: Nature **131**, 56 (1933).
COOK, J. W., DODDS, E. C., HEWETT, C. L., AND LAWSON, W.: Proc. Roy. Soc. London **114B**, 272 (1934).
(39) COOK, J. W., AND DODDS, E. C.: Nature **131**, 205 (1933).
COOK, J. W. (in discussion on tumors): Proc. Roy. Soc. London **113B**, 273 (1933).
(40) COOK, J. W., AND HEWETT, C. L.: Chemistry & Industry **52**, 451 (1933); J. Chem. Soc. **1933**, 1098.
(41) CROFTAN, A. C.: Am. J. Med. Sci. **123**, 150 (1902); Pflüger's Arch. **90**, 635 (1902).
(42) CROWE, J. S.: J. Biol. Chem. **4**, xxxv (1908).
(43) CUNY, L.: Le dosage des sels biliaires dans la bile, etc., Paris, 1930.
(44) DANE, E., AND WIELAND, H.: Z. physiol. Chem. **194**, 119 (1931). Wieland, Paper No. 32.
(45) DEMARÇAY, H.: Ann. **27**, 270 (1833).
(46) DIELS, O., AND ABDERHALDEN, E.: Ber. **36**, 3177 (1903); **37**, 3092 (1904).
(47) DIELS, O.: Ber. **58**, 1231 (1925); **60**, 140 (1927).
DIELS, O., GÄDKE, W., AND KÖRDING, P.: Ann. **459**, 1 (1927).
(48) DIELS, O., AND KARSTENS, A.: Ann. **478**, 136 (1930).
(49) DIELS, O.: Ber. **66**, 487 (1933).
(50) DIELS, O.: Ber. **66**, 1122 (1933).
(51) Discussion on the Chemistry of Sterols: Chemistry & Industry **52**, 10 (1933).
(52) Discussion on the Chemistry of Oestrin: Chemistry & Industry **52**, 268, 287 (1933).
(54) DORÉE, CH., AND GARDNER, J. A.: J. Chem. Soc. **93**, 1328 (1908).
(55) DORÉE, CH., AND GARDNER, J. A.: J. Chem. Soc. **93**, 1625 (1908).
(56) DORÉE, CH.: J. Chem. Soc. **95**, 638 (1909).

(57) DORÉE, CH., AND ORANGE, L.: J. Chem. Soc. **109,** 46 (1916).
(58) DOUBILET, H.: Private communication.
(59) DOUMER, E., AND DOUMER, E.: Compt. rend. soc. biol. **84,** 393, 683 (1921).
(60) DUCO, C. L., AND PANZA, P. T.: Semana méd. **37, I,** 1193 (1930).
(61) DUNOÜY, P. LECOMTE: Surface Equilibria of Biological and Organic Colloids. New York (1926).
(62) ENDERLEN, C.: Ann. **75,** 174 (1850).
(63) ETTISCH, C., AND KOGANEI, R.: Biochem. Z. **193,** 390 (1928).
(64) FERNHOLZ, E.: Ann. **507,** 128 (1933); **508,** 215 (1934).
(65) FISCHER, H.: Z. physiol. Chem. **73,** 204 (1911).
(66) FLINT, AU., JR.: Am. J. Med. Sci. **44,** 305 (1862).
(67) FOSTER, M. G., AND HOOPER, C. W.: J. Biol. Chem. **38,** 355 (1919).
(68) FOSTER, M. G., HOOPER, C. W., AND WHIPPLE, A.: J. Biol. Chem. **38,** 379 (1919).
(69) FOWWEATHER, F. S.: Biochem. J. **26,** 165 (1932).
(70) FRANK, R. T., AND GOLDBERGER, M. A.: Private communication.
(71) FRANK, R. T., AND SOBOTKA, H.: Proc. Soc. Exptl. Biol. Med. **29,** 1026 (1932).
(72) FRENKEL, H., AND CLUZET, J.: Compt. rend. soc. biol. **52,** 1086 (1900).
(73) FÜRTH, O., AND SCHOLL, R.: Biochem. Z. **222,** 430 (1930).
FÜRTH, O., AND MINIBECK, K.: Biochem. Z. **237,** 139 (1931).
(74) GAIS, E.: Proc. Soc. Exptl. Biol. Med. **29,** 216 (1932).
(75) GIRARD, A.: Bull. soc. chim. biol. **15,** 562 (1933).
(76) GO, Y., AND KRATKY, O.: Z. physik. Chem. **26B,** 439 (1934).
(77) GOFF, M., HRDINA, L., AND ANDREWS, E.: Proc. Soc. Exptl. Biol. Med. **29,** 549 (1932).
(78) GOIFFON, R.: Bull. soc. chim. biol. **8,** 165 (1926).
(79) GRASSMANN, W., AND BASU, K. P.: Z. physiol. Chem. **198,** 193 (1931).
(80) GREGORY, R., AND PASCOE, P. A.: J. Biol. Chem. **83,** 35 (1929).
(81) GSELL-BUSSE, M. A.: Arch. exptl. Path. Pharmakol. **138,** 328 (1929).
(82) GUITERAS, A.: Ann. **494,** 116 (1932).
(83) GUITERAS, A.: Z. physiol. Chem. **214,** 89 (1933).
(84) GULLBRING, A.: Z. physiol. Chem. **45,** 448 (1905).
(85) GUNDELACH, C., AND STRECKER, A.: Ann. **62,** 205 (1847).
(86) HAMMARSTEN, H.: Biochem. Z. **147,** 481 (1924).
(87) HAMMARSTEN, O.: Ber. **14,** 71 (1881).
(87a) HAMMARSTEN, O.: Z. physiol. Chem. **24,** 322 (1898).
(88) HAMMARSTEN, O.: Z. physiol. Chem. **61,** 454 (1909).
(89) HARPER, S. H., KON, G. A. R., AND RUZICKA, F. C. J.: J. Chem. Soc. **1934,** 124.
(90) HARRIES, C.: Ber. **45,** 936, 943 (1912).
(91) HAWORTH, R. D., ET AL.: J. Chem. Soc. **1932,** 1125, 1784, 2248; **1933,** 1012.
(92) HAY, M.: in L. Landois and Stirling's Textbook of Human Physiology, 2nd American edition, p. 294. Philadelphia (1886).
(93) HEILBRON, I. M., AND SEXTON, W. A.: J. Chem. Soc. **1929,** 2255.
(94) HEILBRON, I. M., SIMPSON, J. C. E., AND SPRING, F. S.: J. Chem. Soc. **1933,** 626.
(95) HENRIQUES, O. M.: Acta Path. Microbiol. Scand., Supplement **3,** 141 (1930).
(96) HERZOG, R. H., KRATKY, O., AND KURIYAMA, S.: Naturwissenschaften **19,** 524 (1931).
(97) HUECKEL, W., DANNEEL, R., SCHWARTZ, A., AND GEHRKE, A.: Ann. **474,** 121 (1929).

(98) HÜTTIG, G. F.: Z. anorg. Chem. **114**, 24 (1920).
(99) INHOFFEN, H. H.: Ann. **497**, 130 (1932).
(100) INOUYE, K., AND ITO, H.: Z. physiol. Chem. **57**, 313 (1908).
(101) JACOBS, W. A., AND FLECK, E. E.: J. Biol. Chem. **97**, 57 (1932).
(102) JENKE, M.: Arch. exptl. Path. Pharmakol. **130**, 280 (1928).
(103) JOLLES, A.: Z. physiol. Chem. **57**, 30 (1908).
(104) JOSEPHSON, B.: Biochem. Z. **263**, 428 (1933).
(105) KENNAWAY, E. L., AND COOK, J. W.: Chemistry & Industry **51**, 521 (1932).
(106) KISHNER, N.: J. Russ. Phys. Chem. Soc. **44**, 861 (1912).
(107) KOLDA, J.: Compt. rend. soc. biol. **94**, 216 (1928).
(108) KON, G. A. R.: J. Chem. Soc. **1933**, 1081.
(109) KOTAKE, M.: Ann. **465**, 1 (1928).
(110) KUHN, R., AND WASSERMANN, A.: Helv. Chim. Acta **11**, 31 (1928).
(111) LACHINOV (LATSCHINOFF), P. A.: Quoted from Ber. **12**, 1518 (1879).
(112) LACHINOV, P. A.: Ber. **13**, 1911 (1880).
(113) LACHINOV, P. A.: Ber. **15**, 713 (1882).
(114) LACHINOV, P. A.: Ber. **18**, 3039 (1885).
(115) LACHINOV, P. A.: Ber. **19**, 521 (1886).
(116) LACHINOV, P. A.: Ber. **20**, 1053 (1887).
(117) LANDSTEINER, K.: Z. physiol. Chem. **19**, 285 (1894).
(118) LAPWORTH, A., AND CHAPMAN, E. M.: J. Chem. Soc. **75**, 1003 (1899).
(119) LETSCHE, E.: Z. physiol. Chem. **61**, 215 (1909).
(120) LIEB, H.: Mikrochemie **9**, 105 (1931).
(121) LIFSCHÜTZ, J.: Z. physiol. Chem. **50**, 436 (1906); **91**, 309 (1914).
(122) MACLEAN, I. S.: Biochem. J. **22**, 22 (1928).
(123) MCCORQUODALE, D. W., THAYER, S. A., AND DOISY, E. A.: J. Biol. Chem. **99**, 327 (1933).
(124) MCCORQUODALE, D. W., LEVIN, L., AND THAYER, S. A.: J. Biol. Chem. **105**, lv (1934).
(125) MAKINO, H.: Z. physiol. Chem. **220**, 49 (1933).
(126) MARRIAN, G. F.: Biochem. J. **23**, 1090 (1929).
(127) MARRIAN, G. F., AND HASLEWOOD, G. A. D.: Chemistry & Industry **51**, 277 (1932).
(128) MARRIAN, G. F.: Physiol. Rev. **13**, 185 (1933); Science Progress **109**, 69 (1933).
(129) MARSSON, TH.: Arch. Pharm. **58**, 138 (1849).
(130) MAUTHNER, L.: Monatsh. **28**, 1113 (1907); **30**, 635 (1909).
(131) MAZZA, F. P., AND STOLFI, G.: Chim. ind. agr. biol. **8**, 408 (1932).
(132) MENDEL, L. B., AND DANIELS, A. L.: J. Biol. Chem. **13**, 71 (1912).
(133) MENSCHICK, W., PAGE, I. H., AND BOSSERT, K.: Ann. **495**, 225 (1932).
(134) MICHAELIS, L., AND MIZUTANI, M.: Z. physik. Chem. **116**, 135 (1925); **118**, 310 (1927).
(135) MYLIUS, F.: Ber. **19**, 369 (1886).
(135a) MYLIUS, F.: Ber. **20**, 1968 (1887).
(136) NEWMAN, C. E.: Beitr. path. Anat. **86**, 187 (1931).
(137) OKADA, S.: J. Physiol. **50**, 114 (1916).
(138) OTTENBERG, R.: Private communication.
(139) OTTENBERG, R., AND KAHN, J.: Proc. Soc. Exptl. Biol. Med. **29**, 573 (1932).
(140) PAGE, I. H.: J. Biol. Chem. **57**, 471 (1923).
(141) PAGE, I. H., AND MUELLER, EU.: Z. physiol. Chem. **204**, 13 (1931).
(142) PETTENKOFER, M.: Ann. **52**, 90 (1844).
(143) PICKARD, R. H., AND YATES, J.: J. Chem. Soc. **95**, 638 (1908).

(144) PRINGSHEIM, H.: Ber. **48**, 1324 (1915).
(145) PRYDE, J.: Nature **133**, 237 (1934).
(146) QUAGLIARELLO, G.: Atti accad. Lincei **16**, 387 (1932); International Physiological Congress, Rome, 1932.
(147) RAUDNITZ, H., PETRŮ, F., AND HAUROWITZ, F.: Z. physiol. Chem. **209**, 103 (1932).
RAUDNITZ, H., PETRŮ, F., AND STADLER, A.: Ber. **66**, 879 (1933).
(148) REIMANN, A. K., AND MINOT, A. S.: J. Biol. Chem. **45**, 139 (1921).
(149) REINDEL, F., AND WEICKMANN, A.: Ann. **478**, 86 (1929).
(150) REINDEL, F., AND KIPPHAN, H.: Ann. **493**, 181 (1932).
(151) REINHOLD, J. G., AND FERGUSON, L. K.: J. Exptl. Med. **49**, 681 (1929).
(152) REINHOLD, J. G., AND WILSON, D. W.: J. Biol. Chem. **96**, 637 (1932).
(153) RHEINBOLDT, H.: J. prakt. Chem. **111**, 242 (1925).
RHEINBOLDT, H., AND KIRCHEISEN, M.: J. prakt. Chem. **113**, 348 (1926).
RHEINBOLDT, H.: Ann. **451**, 256 (1926); Z. physiol. Chem. **180**, 180 (1928).
RHEINBOLDT, H., KÖNIG, O., AND OTTEN, R.: Ann. **473**, 249 (1929).
(154) RHEINBOLDT, H., KÖNIG, O., AND FLUME, E.: Z. physiol. Chem. **184**, 219 (1929).
(155) ROSENHEIM, O., AND KING, H.: Chemistry & Industry **51**, 464 (1932); Nature **13**, 315 (1932). The Ring System of Sterols and Bile Acids. Paper No. 1.
(156) ROSENHEIM, O., AND KING, H.: Chemistry & Industry **51**, 954 (1932). Paper No. 2.
(157) ROSENHEIM, O., AND KING, H.: Chemistry & Industry **52**, 299 (1933). Paper No. 3.
(158) ROSENHEIM, O., AND KING, H.: Chemistry & Industry **53**, 91 (1934). Paper No. 4.
(159) ROSENHEIM, O., AND KING, H.: Chemistry & Industry **53**, 196 (1934). Paper No. 5.
(160) ROSENHEIM, O., AND STARLING, W. W.: Chemistry & Industry **53**, 1056 (1933).
(161) ROSENHEIM, O., AND KING, H.: Ann. Rev. Biochem. **3**, 87 (1934).
(162) ROUS, P., AND MACMASTER, P. D.: J. Exptl. Med. **34**, 47 (1921).
(163) RUZICKA, L., AND RUDOLPH, E. A.: Helv. Chim. Acta **10**, 916 (1927).
(164) RUZICKA, L., AND THOMANN, G.: Helv. Chim. Acta **16**, 216 (1933).
RUZICKA, L., GOLDBERG, M. W., AND THOMANN, G.: Helv. Chim. Acta **16**, 812 (1933).
(165) SANDQVIST, H., AND GORTON, J.: Ber. **63**, 1935 (1930).
SANDQVIST, H., AND BENGTSSON, E.: Ber. **64**, 2167 (1931).
(166) SCHENCK, M.: Zur Kenntnis der Gallensäuren. Paper No. 4, Z. physiol. Chem. **89**, 360 (1914). Paper No. 5, Z. physiol. Chem. **104**, 284 (1919). Paper No. 6, Z. physiol. Chem. **107**, 152 (1919).
SCHENCK, M., AND KIRCHHOF, H.: Paper No. 16, Z. physiol. Chem. **166**, 142 (1927). Paper No. 32, Z. physiol. Chem. **214**, 42 (1933).
(166a) SCHENCK, M.: Paper No. 7, Z. physiol. Chem. **110**, 167 (1920).
(167) SCHENCK, M.: Paper No. 11, Z. physiol. Chem. **139**, 39 (1924).
(168) SCHMIDT, C. L. A., AND DART, A. E.: J. Biol. Chem. **45**, 415 (1920).
(169) SCHÖNHEIMER, R., ANDREWS, E., AND HRDINA, L.: Z. physiol. Chem. **208**, 182 (1932).
(170) SEKITOO, T.: Z. physiol. Chem. **199**, 225 (1931).
(171) SEYDERHELM, R., TAMMANN, H., AND BAUMANN, W.: Z. exptl. Med. **66**, 539 (1929).

(172) SHIMIZU, T., AND HATAKEYAMA, T.: Z. physiol. Chem. **182**, 57 (1929).
(173) SHIMIZU, T., ODA, T., AND MAKINO, H.: Z. physiol. Chem. **213**, 136 (1932).
(174) SIGNER, R.: Ann. **478**, 246 (1930).
(175) SOBOTKA, H., AND GOLDBERG, A.: Biochem. J. **26**, 555, 557, 566 (1932).
(176) SOBOTKA, H.: Naturwissenschaften **19**, 595 (1931).
SOBOTKA, H., AND GOLDBERG, A.: Biochem. J. **26**, 905 (1932).
(177) SOBOTKA, H., AND KAHN, J.: Biochem. J. **26**, 898 (1932); Ber. **65**, 227 (1932).
(178) SOBOTKA, H.: In Harrow-Sherwin's Biochemistry, Chap. IV. Philadelphia (1935).
(179) STEPHAN, R.: Münch. med. Wochschr. **76**, 1579 (1929).
(180) STOCKHOLM, M., AND SCHMIDT, C. L. A.: Proc. Soc. Exptl. Biol. Med. **30**, 877 (1933).
(181) SZILLARD, P.: Biochem. Z. **159**, 325 (1925); **173**, 440 (1926).
(182) TANRET, C.: Compt. rend. **147**, 75 (1908).
(183) THUDICHUM, J. L. W.: Ann. Clin. Chem. **2**, 251 (1881).
(184) TSCHESCHE, R.: Z. physiol. Chem. **203**, 263 (1932).
(185) TSCHESCHE, R.: Ann. **498**, 185 (1932).
(186) UDRANSKY, L. VON: Z. physiol. Chem. **12**, 355 (1888).
(187) URAKI, Z.: Z. physiol. Chem. **207**, 16 (1932).
(188) VERZAR, F., AND KUTHY, A.: Biochem. Z. **205**, 369 (1929); **210**, 265 (1929).
(189) VERZAR, F.: Klin. Wochschr. **12**, 489 (1933).
(190) VESTERBERG, A.: Ber. **36**, 4200 (1903).
(191) VITALI: L'Orosi **3**, 397 (1881).
(192) VOCKE, F.: Z. physiol. Chem. **191**, 83 (1930).
(193) VORLÄNDER, D.: Ann. **345**, 155, 192 (1906).
(194) WAHLGREN, V.: Z. physiol. Chem. **46**, 556 (1902).
(195) WALLACH, O.: Ann. **312**, 171 (1899).
(196) WALLACH, O.: Ann. **331**, 324 (1902).
(197) WALLACH, O.: Ann. **356**, 231 (1907).
(198) WESTPHALEN, TH.: Ber. **48**, 1064 (1915). Windaus, Paper No. 22.
(199) WIELAND, HEINRICH, AND WEIL, F. J.: Z. physiol. Chem. **80**, 287 (1912). Untersuchungen über die Gallensäuren. Paper No. 1.
(200) WIELAND, H., AND SORGE, H.: Z. physiol. Chem. **96**, 1 (1916). Paper No. 2.
(201) WIELAND, H., AND SORGE, H.: Z. physiol. Chem. **98**, 59 (1916). Paper No. 3.
(202) WIELAND, H., AND STENDER, H.: Z. physiol. Chem. **106**, 181 (1919). Paper No. 4.
(203) WIELAND, H., AND BORSCH, E.: Z. physiol. Chem. **106**, 190 (1919). Paper No. 5.
(204) WIELAND, H., AND KULENKAMPFF, A.: Z. physiol. Chem. **108**, 295 (1920). Paper No. 6.
(205) WIELAND, H.: Z. physiol. Chem. **108**, 306 (1920). Paper No. 7.
(206) WIELAND, H., AND WEYLAND, P.: Z. physiol. Chem. **110**, 123 (1920). Paper No. 8.
(207) WIELAND, H., AND SCHULENBURG, W.: Z. physiol. Chem. **114**, 167 (1921). Paper No. 10.
(208) WIELAND, H., AND SCHLICHTING, O.: Z. physiol. Chem. **119**, 76 (1922). Paper No. 11.
(209) WIELAND, H., AND SCHLICHTING, O.: Z. physiol. Chem. **120**, 227 (1922). Paper No. 12.
(210) WIELAND, H., AND ADICKES, F.: Z. physiol. Chem. **120**, 232 (1922). Paper No. 13.

(211) WIELAND, H., AND SCHLICHTING, O.: Z. physiol. Chem. **123**, 213 (1922). Paper No. 14.
(212) WIELAND, H., AND FUKELMAN, L.: Z. physiol. Chem. **130**, 144 (1923). Paper No. 16.
(213) WIELAND, H., HONOLD, E., AND PASCUAL-VILLA, J.: Z. physiol. Chem. **130**, 326 (1923). Paper No. 17.
(214) WIELAND, H., FLASCHENTRÄGER, B., AND REWOLLE, A.: Z. physiol. Chem. **134**, 140 (1924). Paper No. 18.
(215) WIELAND, H., AND MOTHES, W.: Z. physiol. Chem. **134**, 149 (1924). Paper No. 19.
(216) WIELAND, H., AND SCHLICHTING, O.: Z. physiol. Chem. **134**, 276 (1924). Paper No. 20.
(217) WIELAND, H., AND REVEREY, G.: Z. physiol. Chem. **140**, 186 (1924). Paper No. 21.
(218) WIELAND, H., AND JACOBI, R.: Z. Physiol. Chem. **148**, 232 (1925). Paper No. 23.
(219) WIELAND, H., AND SCHLICHTING, O.: Z. physiol. Chem. **150**, 267 (1925). Paper No. 24.
(220) WIELAND, H., SCHLICHTING, O., AND LANGSDORFF, W. VON: Z. physiol. Chem. **161**, 74 (1926). Paper No. 25.
(221) WIELAND, H., SCHLICHTING, O., AND JACOBI, R.: Z. physiol. Chem. **161**, 80 (1926). Paper No. 26.
(222) WIELAND, H.: Z. physiol. Chem. **167**, 70 (1927). Paper No. 27.
(223) WIELAND, H., AND VOCKE, F.: Z. physiol. Chem. **177**, 68 (1929). Paper No. 28.
(224) WIELAND, H., AND VOCKE, F.: Z. physiol. Chem. **191**, 69 (1930). Paper No. 30.
(225) WIELAND, H., ERTEL, L., AND DANE, E.: Z. physiol. Chem. **194**, 107 (1930). Paper No. 31.
(226) WIELAND, H., AND POSTERNAK, TH.: Z. physiol. Chem. **197**, 17 (1931). Paper No. 34.
(227) WIELAND, H., AND DANE, E.: Z. physiol. Chem. **206**, 225 (1932). Paper No. 37.
(228) WIELAND, H., AND DANE, E.: Z. physiol. Chem. **206**, 243 (1932). Paper No. 38.
(229) WIELAND, H., AND DANE, E.: Z. physiol. Chem. **210**, 268 (1932). Paper No. 39.
(230) WIELAND, H., AND KRAFT, K.: Z. physiol. Chem. **211**, 203 (1932). Paper No. 42.
(231) WIELAND, H., DANE, E., AND SCHOLZ, E.: Z. physiol. Chem. **211**, 261 (1932). Paper No. 43.
(232) WIELAND, H., AND DANE, E.: Z. physiol. Chem. **212**, 41 (1932). Paper No. 44.
(233) WIELAND, H., AND DANE, E.: Z. physiol. Chem. **212**, 263 (1932). Paper No. 45.
(234) WIELAND, H., AND KISHI, S.: Z. physiol. Chem. **214**, 47 (1933). Paper No. 47.
(235) WIELAND, H., AND DANE, E.: Z. physiol. Chem. **216**, 91 (1933). Paper No. 50.
(236) WIELAND, H., AND DANE, E.: Z. physiol. Chem. **219**, 240 (1933). Paper No. 52.
(237) WIELAND, H., AND ALLES, R.: Ber. **55**, 1789 (1922).
(238) WIELAND, H., AND JACOBI, R.: Ber. **59**, 2064 (1926).
(239) WIELAND, H., STRAUB, H., AND DORFMÜLLER, TH.: Z. physiol. Chem. **186**, 97 (1930).
(240) WIELAND, H., AND VOCKE, F.: Ann. **481**, 215 (1930).
(241) WIELAND, H., HESSE, G., AND MEYER, H.: Ann. **493**, 272 (1932).
(242) WIELAND, HERMANN, AND HILDEBRAND, TH.: Arch. exptl. Path. Pharmakol. **85**, 199 (1920).
WIELAND, HERMANN: Arch. exptl. Path. Pharmakol. **86**, 79, 92 (1920).

(243) WILLSTÄTTER, R., AND MEYER, E.: Ber. **41**, 2199 (1908).
(244) WINDAUS, A.: Ber. **37**, 2027 (1904). Über Cholesterin. Paper No. 2.
(245) WINDAUS, A.: Ber. **39**, 2008 (1906). Paper No. 6.
(246) WINDAUS, A.: Ber. **41**, 2558 (1908). Paper No. 11.
(247) WINDAUS, A.: Ber. **42**, 3770 (1909). Paper No. 12.
(248) WINDAUS, A., AND RESAU, C.: Ber. **46**, 1246 (1913). Paper No. 16.
(249) WINDAUS, A., AND UIBRIG, CL.: Ber. **47**, 2384 (1914). Paper No. 19.
(250) WINDAUS, A., AND UIBRIG, CL.: Ber. **48**, 857 (1915). Paper No. 21.
(251) WINDAUS, A.: Ber. **49**, 1724 (1916). Paper No. 24.
(252) WINDAUS, A., AND NEUKIRCHEN, K.: Ber. **52**, 1955 (1919). Paper No. 28.
(253) WINDAUS, A., AND KIRCHNER, E.: Ber. **53**, 614 (1920). Paper No. 30.
(254) WINDAUS, A.: Ber. **42**, 238, 616 (1909).
(255) WINDAUS, A., AND STADEN, A. VON: Z. physiol. Chem. **117**, 147 (1921).
(256) WINDAUS, A., HÜCKEL, W., AND REVEREY, G.: Ber. **56**, 91 (1923).
(257) WINDAUS, A., AND BOHNE, A.: Ann. **433**, 278 (1923).
(258) WINDAUS, A., AND HAUTH, A.: Ber. **39**, 4378 (1906); **40**, 3681 (1907).
WINDAUS, A., AND BRUNKEN, I.: Z. physiol. Chem. **140**, 47 (1924).
(259) WINDAUS, A., BOHNE, A., AND SCHWARZKOPF, E.: Z. physiol. Chem. **140**, 177 (1924).
(260) WINDAUS, A., AND HOSZFELD, R.: Z. physiol. Chem. **145**, 177 (1925).
(261) WINDAUS, A., AND VAN SCHOOR, A.: Z. physiol. Chem. **148**, 225 (1925).
(262) WINDAUS, A., AND BOHNE, A.: Ann. **442**, 7 (1925).
(263) WINDAUS, A.: Ann. **447**, 233 (1926).
(264) WINDAUS, A., AND VAN SCHOOR, A.: Z. physiol. Chem. **173**, 312 (1928).
(265) WINDAUS, A., BERGMANN, W., AND KÖNIG, G.: Z. physiol. Chem. **189**, 148 (1930).
(266) WINDAUS, A., AND TSCHESCHE, R.: Z. physiol. Chem. **190**, 51 (1930).
(267) WINDAUS, A.: Z. physiol. Chem. **213**, 147 (1932).
(268) WOLFF: Ann. **394**, 86 (1912).

(269) LETTRÉ, H.: Ann. **511**, 280 (1934).
(270) CIAMICIAN, G., AND SILBER, P.: Ber. **41**, 1071 (1908).
(271) CHEN, K. K., AND CHEN, A. L.: Arch. intern. pharmacodynamie **47**, 297 (1934).
(272) FELS, E., AND SLOTTA, K. H.: Klin. Wochschr. **10**, 1639 (1931).
ALLEN, W. M.: J. Biol. Chem. **98**, 591 (1932).
FEVOLD, H. L., AND HISAW, F. L.: Proc. Soc. Exptl. Biol. Med. **29**, 620 (1932).
(273) BUTENANDT, A., WESTPHAL, U., ET AL.: Z. physiol. Chem. **227**, 84 (1934); Ber. **67**, 1440, 1893, 1897, 2088, 2092 (1934).
WINTERSTEINER, O., AND ALLEN, W. M.: J. Biol. Chem. **107**, 321 (1934).
SLOTTA, K. H., RUSCHIG, H., AND FELS, E.: Ber. **67**, 1270 (1934).
HARTMANN, M., AND WETTSTEIN, A.: Helv. Chim. Acta **17**, 878 (1934).
(274) BUTENANDT, A., AND SCHMIDT, J.: Ber. **67**, 1901 (1934).
(275) BUTENANDT, A., WESTPHAL, U., AND COBLER, H.: Ber. **67**, 1611, 2085 (1934).
FERNHOLZ, E., AND CHAKRAVORTY, P. N.: Ber. **67**, 1855, 2021, 2027 (1934).
(276) BUTENANDT, A. AND TSCHERNING, H.: Z. physiol. Chem. **229**, 167, 185, 192 (1934).
(277) RUZICKA, L., GOLDBERG, M. W., ET AL.: Helv. Chim. Acta **17**, 1389, 1395 (1934).
(278) CHARGAFF, E., AND ABEL, G.: Biochem. J. **28**, 1901 (1934).
(279) WINTERSTEIN, A., AND SCHÖN, K.: Naturwissenschaften **22**, 237–8 (1934).