Chemistry and Biochemistry of Some Mammalian Secretions and Excretions.

A CENTENARY LECTURE DELIVERED BEFORE THE CHEMICAL SOCIETY AT THE ROYAL INSTITUTION ON MAY 12TH, 1949.

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DURING the last nine years we have studied in detail the chemical composition of different animal secretions and excretions. The result of this work seems to present a certain interest, not only from the point of view of the particular drug or material studied, but also from a larger, biochemical point of view. We have isolated a certain number of new substances, the constitutions of which were determined whenever possible; we have tried to find satisfactory biochemical explanations for the presence or formation of these substances in the animal. The following materials were studied: (1) the scent gland of the beaver; (2) pregnant mare's urine; (3) wool fat; and (4) ambergris.

(1) THE SCENT GLAND OF THE BEVER (CASTOREUM).

The dried scent glands of the Canadian beaver (*Castor fiber*) are commercially known under the name castoreum and are still utilized in perfumery because of the strong animal odour of their extracts. The beaver of either sex has two of these pearshaped organs in the anal region, which weigh about 25—50 g. each. Their secretion is voided into the cloaca, and the beaver is said to leave an odorous trail which allows the animals to find one another.

The only papers on the chemical composition of the scent glands of the beaver were those of Walbaum and Rosenthal (*J. pr. Chem.*, 1927, **117**, 225) and of Pfau (*Perf. and Ess. Oil Rec.*, 1927, **18**, 206) describing the isolation of benzyl alcohol, borneol, *p*-ethylphenol, acetophenone, benzoic and salicylic acids. We have confirmed all these findings and have isolated about forty other substances, some of which have never before been found in Nature, others which have never been described at all; still others are awaiting isolation and identification.

Table I lists about forty of these compounds. It is certainly very astonishing to find so many unusual, mostly aromatic substances in an animal gland. In the course of our work we

TABLE I.

Constituents of castoreum (scent glands of the Canadian beaver).

Alcohols:	benzyl alcohol (free and esterified), cholesterol, β-cholestanol, mannitol (*U*), *cis*-5-hydroxytetrahydroionol (*U*).
Phenols:	*p*-ethylphenol (*U*, *b*), *p*-propylphenol (*b*), pyrocatechol (*U*), quinol (*U*), quinol monomethyl ether (*a*), chavicol (*a*), 2-hydroxy-5-ethylanisole (*a*), 4-methyl- (*a*) and 4-ethyl-pyrocatechol (*a*), betuligenol (*a*), 2 : 4′-dihydroxydiphenylmethane (*b*), 2′ : 3″-dihydroxydibenz-2-pyrone (pigment I, *c*), 4 : 4′-dihydroxydiphenic acid dilactone (pigment II, *c*); a phenolic ether, m. p. 83° (*c*).
Aldehydes:	salicylaldehyde (*a*).
Ketones:	acetophenone (*a*), *p*-hydroxyacetophenone (*a*), *p*-methoxyacetophenone (*b*), aromatic ketone $C_{10}H_{12}O_2$, two isomeric hydroxy-ketones $C_{13}H_{24}O_2$ probably derived from ionone (*U*, *b*).
Acids:	benzoic (*U*), β-phenylpropionic (*a*), cinnamic (*U*), salicylic (*U*), *m*-hydroxybenzoic (*U*), *p*-hydroxybenzoic (*U*), anisic (*a*), gentisic (*U*), 5-methoxysalicylic (*b*).
Esters:	cholesterol oleate, a phenolic ester (m. p. 170°), esters of ceryl and benzyl alcohol, and of phenols and gentisic acid.
Amines:	castoramine, $C_{15}H_{23}O_2N$, m. p. 66° (*c*).

(*U*), Normally found in urine. (*a*) Isolated for the first time from an animal organ.
(*b*) Isolated for the first time from a natural source. (*c*) New compound.

came to the conclusion that two main reasons explain this. First, the special food habits of the beaver: his food consists mainly of buds and barks of trees; these plant tissues contain a great number of substances which we have found also in the scent glands (borneol, pyrocatechol, betuligenol, *p*-hydroxyacetophenone, salicylaldehyde, benzoic, cinnamic, and salicylic acids, etc.). In other vertebrates, such substances, ingested with the food, would be excreted in the urine after appropriate conjugation. The *beaver*, on the contrary, *deposits these in his scent*

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glands, without conjugation; this is the second reason which explains the extraordinary composition of this secretion. The numerous substances of Table I, marked (*U*) are those which are normal constituents of vertebrate urine.

Some substances are deposited unchanged, some after hydrolysis of the glycosidic linkage (piceoside ⟶ *p*-hydroxyacetophenone; betuloside ⟶ betuligenol), others after oxidation (salicylic acid ⟶ gentisic acid) or reduction (xanthophylls ⟶ tetrahydroionones; cinnamic acid ⟶ β-phenylpropionic acid).

We shall next discuss in greater detail the chemistry and biochemistry of some of the constituents of castoreum.

Mannitol. In a recent note on the constituents of castoreum (*Nature*, 1946, **157**, 231) we mentioned a " water-soluble nitrogen-free substance melting at 173°." We have since identified it as D-mannitol, which is a normal constituent of dog's urine (Kapfhammer and Prell, *Biochem. J.*, 1937, **295**, 91) but seems not to have been found before in an animal organ.

Acetophenone. The presence of rather large amounts of this ketone, which is found only in very small amounts in some essential oils, can be explained by a mechanism described by Dakin (*J. Biol. Chem.*, 1909, **6**, 217; **9**, 123) in experiments involving administration of β-phenylpropionic acid to cats and dogs; this acid is oxidized at the β-carbon and then decarboxylated to acetophenone, which was found in the urine.

$$Ph{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H \longrightarrow OH{\cdot}CHPh{\cdot}CH_2{\cdot}CO_2H \longrightarrow Ph{\cdot}CO{\cdot}CH_2{\cdot}CO_2H \longrightarrow Ph{\cdot}CO{\cdot}CH_3$$

Gentisic acid, which is one of the major acids of castoreum, is the normal oxidation product of salicylic acid in the vertebrates and is usually found in the urine after administration of salicylic acid (Kapp and Coburn, *J. Biol. Chem.*, 1942, **145**, 549; Lutwak-Mann, *Biochem. J.*, 1943, **37**, 246). However, it had not previously been found in an animal tissue.

Betuligenol is a phenol, $C_{10}H_{14}O_2$, m. p. 80°, the constitution of which we established in 1941, independently of Sosa (*Ann. Chim.*, 1940, **11**, 1) who had isolated it as an aglycone of betuloside extracted from the bark of the birch. Oxidation of the monomethyl ether of betuligenol with permanganate gave anisic acid, and chromic acid oxidation gave 1-*p*-methoxyphenylbutan-3-one, identical with the synthetic product. Betuligenol must thus be 1-*p*-hydroxyphenylbutan-3-ol, one of the rare natural aromatic substances with a butyl side chain (Lederer and Polonsky, *Bull. Soc. Chim. biol.*, 1942, **24**, 1386).

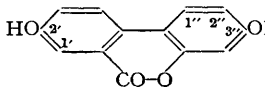
2 : 4′-*Dihydroxydiphenylmethane*, m. p. 120°, was identified by chromic acid oxidation to 2 : 4′-dihydroxybenzophenone. This is the first time that this substance has been isolated from a natural source.

Pigments of castoreum. The scent glands of the beaver contain two new yellow pigments; they are soluble with an intense yellow colour in aqueous carbonate and can be separated from one another by fractional crystallisation of their colourless acetates (Lederer, *ibid.*, p. 1155).

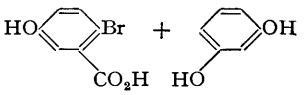
Pigment I, $C_{13}H_8O_4$, can be obtained as yellow needles, melting above 360° (decomp.), by hydrolysis of its diacetate, m. p. 210°. It contains two phenolic hydroxyls and one lactone group. By distillation with zinc powder it is reduced to fluorene, $C_{13}H_{10}$, whereby all 13 carbon atoms are accounted for. Fusion with potassium hydroxide gives *m*-hydroxybenzoic acid. These results, as well as the positive Griessmayer reaction (see below), led us to formulate pigment I as 2′ : 3″-dihydroxydibenzo-2-pyrone (I). We have recently confirmed this structure by synthesis, using the method of Hurtley (*J.*, 1929, 1870). By condensing 2-bromo-5-hydroxybenzoic acid (II) with resorcinol, in the presence of traces of copper sulphate as catalyst, we have obtained a yellow pigment, the diacetate, the dimethyl ether, and the two derivatives (III*a*) and (III*b*), none of which depressed the melting points of the natural derivatives (Lederer and Polonsky, *Bull. Soc. chim.*, 1948, 831). The same method may be used to synthesize various analogues of pigment I.

Pigment II of castoreum is obtained by hydrolysis of its very insoluble diacetate, m. p. 320°. It forms greenish-yellow needles, which do not melt at 360°. Its formula is $C_{14}H_6O_6$ and it contains two phenolic hydroxyl groups and two lactone groups, whereby all six oxygen atoms are accounted for. On distillation with zinc powder, it is also reduced to fluorene, and fusion with potassium hydroxide gives β-resorcylic acid. These results lead to the formula (IV) for this pigment (Lederer, *Bull. Soc. Chim. biol.*, 1942, **24**, 1155). An asymmetrical formula, with only one hydroxyl in a 4-position of the diphenyl group, would however still be compatible with the above-mentioned experimental results. We excluded this possibility by studying Griessmayer's reaction (*Annalen*, 1871, **160**, 51) with similar pigments. This reaction consists in heating ellagic acid (V*a*) or similar pigments with nitrous acid containing nitric acid, whereupon a red colour develops which is stable after dilution with water. We found that only those

pigments which contain at least two free hydroxyl groups in 4 : 4′-positions of the diphenyl nucleus give this reaction. This rule was also found true for the synthetic dibenz-2-pyrones.



(I.)



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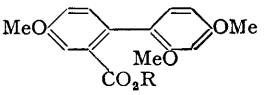
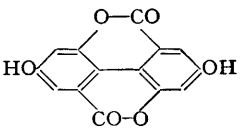


(III*a*; R = H.)
(III*b*; R = Me.)



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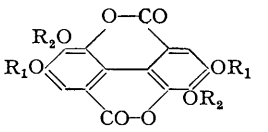
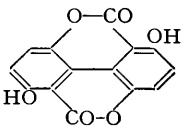


(V*a*; $R_1 = R_2 = H$.)
(V*b*; $R_1 = H$; $R_2 = Me$.)
(V*c*; $R_1 = Me$; $R_2 = H$.)



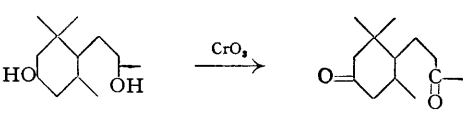
(VI.)

Shinoda and Kun (*J. Pharm. Soc. Japan*, 1931, **51**, 50) have described a dimethyl ether of ellagic acid, m. p. 337°, isolated from the roots of *Euphorbia formosana*, which gives no reaction with ferric chloride but gives a positive Griessmayer reaction. This must therefore be (V*b*), and the isomeric dimethyl ether which Goldschmiedt (*Monatsh.*, 1905, **26**, 1139) prepared by methylation of ellagic acid must be (V*c*).

In the course of this work we became interested in an isomer of pigment II, obtained by Juch (*Monatsh.*, 1905, **26**, 839) by oxidation of gentisic acid with manganese dioxide. The exact structure of this pigment had not been established, but Juch stated that distillation with zinc dust reduced it to phenanthrene (m.p. 100°). On repeating his experiment, we found that his phenanthrene was in reality fluorene (m. p. 116°), and therefore his pigment must have the structure (VI); the Griessmayer reaction of this pigment is of course negative.

Pigment I and, more so, pigment II have a close relation with ellagic acid (V*a*), which is abundant in the bark of trees. It is as yet difficult to tell if these two pigments of castoreum are reduction products of ellagic acid, or if they exist as such in some plant material.

Derivatives of ionone. Prelog and collaborators (*Helv. Chim. Acta*, 1947, **30**, 113; 1948, **31**, 1799) have recently described the isolation and synthesis of a series of very interesting neutral substances with thirteen carbon atoms, isolated from pregnant-mare's urine. These are glycols



(VII.) (VIII.)

(VII), keto-alcohols, and diketones (VIII) derived from tetrahydroionone and having one of the oxygen atoms in the same position as the carbonyl group of ionone and the second oxygen atom on the ring-carbon atom 5, that is, the carbon atom at which the xanthophylls have their hydroxyl group. Prelog and his collaborators think that these substances are derived from the xanthophyll pigments of the food.

In 1943, we described an amorphous glycol, isolated from castoreum (*Bull. Soc. Chim. biol.*, 1943, **25**, 1381) for which we had proposed the formula $C_{12}H_{24}O_2$ of a monocyclic glycol; the oxidation of this glycol had given a diketone (bis-2 : 4-dinitrophenylhydrazone, m. p. 198°) showing the characteristic colour reaction of methyl ketones. Since then, our glycol has crystallized (m. p. 100°) and a later conversation with Professor Prelog bore out the probability that our glycol was identical with the mixture (m. p. 103°) of stereoisomeric diols $C_{13}H_{26}O_2$ isolated in Zürich from pregnant-mare's urine. A comparison of the bisphenylsemicarbazones and the bis-2 : 4-dinitrophenylhydrazones, and of the infra-red spectra of our diketone with that of Prelog's compound confirmed the identity (Lederer, Prelog, and Schneider, *Helv. Chim. Acta*, 1948, **31**, 2133). It is very probable that the two isomeric keto-alcohols $C_{13}H_{24}O_2$ which we had also isolated from castoreum (*Bull. Soc. Chim. biol.*, 1943, **25**, 1073) are identical with corresponding ionone derivatives isolated by Prelog *et al.* We have here a new illustration of the parallelism between castoreum and the urine of vertebrates, and a strong indication that the ionone derivatives of Prelog *et al.* are of general importance as degradation products of carotenoids.

Castoramine is a crystalline base, m. p. 66°, isolated from castoreum (*ibid.*, p. 1299); it has

the formula $C_{15}H_{23}O_2N$ and is characterised by its sparingly soluble sulphate, m. p. 216°. Castoramine contains no methoxyl or methylimide group and no mobile hydrogen. Recently Schinz, Ruzicka, Geyer, and Prelog (*Helv. Chim. Acta*, 1946, **29**, 1524) have isolated from the musk glands of the musk deer a pyridine derivative $C_{16}H_{25}N$, which they called " muscopyridine." Despite the similarity of the formulæ and of the origin of these two " animal alkaloids," castoramine does not seem to be very closely related to muscopyridine, because it has not the characteristic absorption spectrum of pyridine derivatives.*

(2) Pregnant-mare's Urine.

The analogy between the constituents of the scent glands of the beaver and those normally found in urine prompted us to extend our investigation to the phenols and acids of pregnant-mare's urine.

p-*Ethylphenol*. It has been known for more than 50 years that the urine of vertebrates contains the ethereal sulphates of different phenolic substances which are derived from tyrosine by bacterial degradation in the intestine. Baumann had already in 1879 (*Ber.*, 1879, **12**, 1452) proposed the following scheme of degradation of tyrosine: tyrosine ⟶ *p*-hydroxyphenylpropionic acid ⟶ *p*-ethylphenol ⟶ *p*-hydroxyphenylacetic acid ⟶ *p*-cresol ⟶ *p*-hydroxybenzoic acid ⟶ phenol. All these substances had since been isolated from urine of vertebrates, except one, *p*-ethylphenol.

Having confirmed the findings of Walbaum and Rosenthal (*loc. cit.*) and of Pfau (*loc. cit.*) that *p*-ethylphenol is the principal phenol of castoreum and having established the close relation between the constituents of this animal gland and the substances normally excreted by other vertebrates in the urine, we thought that *p*-ethylphenol should also be a normal constituent of vertebrate urine. By a careful fractional distillation of the phenolic non-ketonic portion of an extract of hydrolysed pregnant-mare's urine, we were able to isolate a phenol, the phenylurethane and glycollic acid ether of which did not depress the melting points of derivatives of authentic *p*-ethylphenol (*ibid.*, p. 1237). In pregnant-mare's urine, *p*-ethylphenol amounts only to 4—10% of the *p*-cresol present. In castoreum, on the contrary, we have found no *p*-cresol at all. That there actually exist very definite species differences between the phenols excreted in the urine of different vertebrates is shown by the recent report of Grant (*Biochem. J.*, 1948, **43**, 523) who isolated the ethereal sulphate of *p*-ethylphenol from goat's urine and found *p*-cresol to be present in only very small amounts. Thus castoreum seems to resemble goat's urine closely in this respect.

p-*Propylphenol* had never been isolated from an animal or plant tissue; in castoreum it accompanies *p*-ethylphenol in a proportion of approximately 1 : 10. We have found no *p*-propylphenol in pregnant-mare's urine, but we suppose that it may also be derived in the beaver from tyrosine by bacterial deamination and reduction of the carboxyl group. It is significant that no *p*-butylphenol or higher straight-chain phenol with a *p*-hydroxyl group has been found either in castoreum or in pregnant-mare's urine.

Acids. The presence of *m*-hydroxybenzoic acid in castoreum had surprised us; this acid had only once before been isolated from a natural source. Bielig and Hayasida (*Z. physiol. Chem.*, 1940, **266**, 99) found it in the urine of rabbits which had been dosed with β-ionone. They recognized, of course, that the β-ionone could not have been transformed into *m*-hydroxybenzoic acid, but they did not suspect that it could be a normal constituent of vertebrate's urine.

The numerous analogies, already cited, between the constituents of the scent gland of the beaver and vertebrate's urine led us to suppose that *m*-hydroxybenzoic acid was, in fact, a normal constituent of vertebrate's urine; so we decided to analyse the acids of pregnant-mare's urine which are industrial by-products of the production of the female sex hormones. This study seemed all the more interesting as no such analysis had yet been made in detail.

By fractional distillation of the ethyl esters of 900 g. of acids of pregnant-mare's urine we have isolated fifteen acids (see Table II), thirteen of which have been identified; only four of these had been known until then as normal constituents of vertebrate's urine : benzoic, phenylacetic, *p*-hydroxybenzoic, and azelaic acid (Lederer and Polonsky, *Biochim. Biophys. Acta*, 1948, **2**, 431). The nine new acids are salicylic, vanillic, *m*-hydroxybenzoic (which thus *is* actually a normal constituent of urine), three unsaturated acids [cinnamic, *p*-cumaric (*p*-hydroxycinnamic), and ferulic (4-hydroxy-3-methoxycinnamic) acid], and three acids which had not yet been isolated from a natural source [*m*-methoxybenzoic, dihydroferulic (4-hydroxy-3-methoxyphenylpropionic acid) and decane-1 : 10-dicarboxylic acid] (see Table II).

* We have to thank Professor V. Prelog for measuring the absorption spectrum of castoramine.

TABLE II.

Acids isolated from pregnant-mare's urine.

Acid.	M. p.	% of total acid.	Mg. per l. of urine.	Acid.	M. p.	% of total acid.	Mg. per l. of urine.
Benzoic	122°	74	2460	Substituted salicylic (not identified)	118°	0·07	2·3
Phenylacetic	76	14	460	Ferulic	170	0·04	1·3
p-Hydroxybenzoic	214	1·3	43	Vanillic	205	0·03	0·9
Salicylic	156	0·7	23	*m*-Methoxybenzoic	105	0·025	0·7
Azelaic	107	0·4	13	Cinnamic	134	0·02	0·6
Dihydroferulic	90	0·2	6·6	Phenolic (not identified)	208	0·01	0·3
Decane-1 : 10-dicarboxylic	128	0·1	3·3	*p*-Cumaric	210	0·002	0·06
m-Hydroxybenzoic	197	0·1	3·3				

The acids mentioned thus comprise eleven aromatic and two aliphatic dicarboxylic acids. It is possible that other, more water-soluble acids have been lost during the industrial preparation. As the material investigated had been extracted from hydrolysed urine, we can say nothing about the form of conjugation of these acids in pregnant-mare's urine.

Let us now consider the biochemical implications of these findings.

(*a*) The methoxylated acids, vanillic, ferulic, and dihydroferulic acids, are probably derived from lignin or similar food substances.

(*b*) The isolation of three unsaturated acids, cinnamic, cumaric, and ferulic acid, is worth notice, as it was believed until then that such acids pass into the urine only after administration of large doses. Fischer and Bielig (*Z. physiol. Chem.*, 1940, **266**, 73) found more than half of a dose of several grams of cinnamic acid excreted in the urine of rabbits, whereas Snapper-Yü and Chiang (*Proc. Soc. Exp. Biol. Med.*, 1940, **44**, 30) found only traces of unchanged acid in human urine, after a dose of 6 g. of cinnamic acid. It seems probable that the cinnamic acid derivatives which we have isolated are formed in the organism as intermediates in the oxidation of saturated acids, according to Dakin's well-known scheme (" Oxidations and Reductions in the Animal Body," London, 1922). If this is true, then cinnamic acid is another metabolic product of phenylalanine, and *p*-cumaric acid of tyrosine.

(*c*) Of the two dicarboxylic acids, which we have isolated, azelaic acid has been found by Müller (*J. Biol. Chem.*, 1937, **119**, 121) in the urine of cows, together with pimelic acid. We have not found the latter in pregnant-mare's urine. Azelaic acid, which Hanson (*Ernährung*, 1941, **6**, 273) considers to be a normal constituent of human urine, may easily be formed by oxidation of oleic, linoleic, and linolenic acid, which all have the grouping $X = CH{\cdot}[CH_2]_7{\cdot}CO_2H$, or also, according to Verkade, Van der Lee, and Van Alphen (*Z. physiol. Chem.*, 1937, **250**, 47), from certain acids considered to be " diacidogenic."

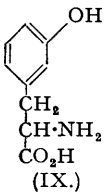
As for the decane-1 : 10-dicarboxylic acid, it has never before been found in Nature. Emmrich and Emmrich-Glaser (*Z. physiol. Chem.*, 1940, **266**, 183) have studied its fate after administration to dogs and humans : 6—7% are excreted unchanged in the urine. It is perhaps formed partly by ω-oxidation of long-chain fatty acids. We wish, however, to discuss another possibility, suggested by a reaction studied by Lapworth and Mottram (*J.*, 1925, **127**, 987) and Green and Hilditch (*J.*, 1937, 764). These authors found that the dihydroxy-acids derived from the natural unsaturated acids (for instance, θι-dihydroxystearic derived from oleic acid, or λμ-dihydroxybehenic derived from erucic acid) are oxidised by permanganate in a peculiar way which is illustrated below for the last-mentioned acid : the carbon chain is cut into three pieces, by rupture next to the two hydroxyl groups; the middle piece gives oxalic acid. Erucic acid being a rather common constituent of natural oils, this mechanism could account for the isolation of decane-1 : 10-dicarboxylic acid.

$$CH_3{\cdot}[CH_2]_7{\cdot}CH{:}CH{\cdot}[CH_2]_{11}{\cdot}CO_2H \longrightarrow CH_3{\cdot}[CH_2]_7{\cdot}CH(OH){\cdot}CH(OH){\cdot}[CH_2]_{11}{\cdot}CO_2H \longrightarrow$$
$$CH_3{\cdot}[CH_2]_6{\cdot}CO_2H + H_2C_2O_4 + HO_2C{\cdot}[CH_2]_{10}{\cdot}CO_2H$$

(*b*) The case of *m*-hydroxybenzoic acid is still mysterious; this acid has as yet not been isolated from plant material, and its formation by oxidation of a *m*-hydroxylated natural substance remains improbable as long as such a substance has not been found. Dakin and Herter showed long ago (*J. Biol. Chem.*, 1907, **3**, 419) that benzoic acid, treated under certain conditions with hydrogen peroxide, is oxidized to a mixture of *o*-, *m*-, and *p*-hydroxybenzoic acids. This mode of formation *in vivo* seems rather improbable in view of recent work on the

metabolism of aromatic substances, where only *o*- or *p*-hydroxylations have been encountered (Williams, *Biochem. J.*, 1946, **40**, 219; 1947, **41**, 1; Bray, Ryman, and Thorpe, *ibid.*, 1947, **41**, 212).

Another possibility was indicated by a recent paper of Blaschko and Stanley (*ibid.*, 1948, **42**, iii) stating that *m*-tyrosine (IX) is a substrate for some enzymes (dopa-decarboxylase of rat liver, and D-amino-oxidase of pig kidney). We thought that if *m*-tyrosine is a substrate for enzymes it is perhaps a naturally occurring amino-acid, formed in small quantity from phenylalanine at the same time as *p*-tyrosine. If that were so, the presence of *m*-hydroxybenzoic acid in urine would be quite natural. A search for *m*-tyrosine in the mother-liquors of *p*-tyrosine and in the blood and urine of rabbits after injection of phenylalanine has so far been negative.* A last possibility, which should be discussed, is that *m*-hydroxybenzoic acid is formed in the intestine by reductive deamination of 3-hydroxyanthranilic acid, which has recently been shown to be an intermediate in the transformation of tryptophan into nicotinic acid (Albert, Scheer, and Deuel, jun., *J. Biol. Chem.*, 1948, **175**, 479; Heidelberger, Abraham, and Lepkovsky, *ibid.*, 1948, **176**, 1461).



(IX.)

As for the *m*-methoxybenzoic acid isolated from pregnant-mare's urine, nothing can be said concerning its origin; methylations of phenolic hydroxyl groups have not yet been observed in the animal organism. It seems to us that this question deserves to be studied again, because such a methylation could account also for the presence of *p*-methoxyacetophenone and 5-methoxysalicylic acid in the scent gland of the beaver (see Table I).

(3) Wool Fat.

It is not our intention to cover here the whole literature of the chemistry of wool fat, the waxy substance excreted by the sebaceous glands of the sheep on to the wool fibre. The reader may consult our recent review on that subject (Lederer and Velluz, *Ind. Parfumerie*, 1947, **2**, 282). We wish to mention here some results of a chromatographic study of the unsaponifiable portion of wool fat and of an investigation into the biochemistry of the triterpene alcohols of wool fat.

Wool fat is essentially a wax, consisting of esters of branched-chain fatty acids (Weitkamp, *J. Amer. Chem. Soc.*, 1945, **67**, 447) with aliphatic and cyclic alcohols. The most important of these latter are cholesterol and "*iso*cholesterol," which is a mixture of four tetracyclic triterpene alcohols (lanosterol, $C_{30}H_{50}O$, dihydrolanosterol, $C_{30}H_{52}O$, agnosterol, $C_{30}H_{48}O$ and dihydroagnosterol, $C_{30}H_{50}O$) which have been studied in recent years by Ruzicka, Denss, and Jeger (*Helv. Chim. Acta*, 1945, **28**, 759; 1946, **29**, 204) and by Dorée, McGhie, and Kurzer (*J.*, 1947, 1467; *J.*, 1948, 988; *Nature*, 1949, **163**, 140). Lanosterol and agnosterol have the same carbon skeleton (Marker, Wittle, and Mixon, *J. Amer. Chem. Soc.*, 1937, **59**, 1368) and their hydroxyl group is at the same carbon atom (2) as in most plant triterpenes (Ruzicka, Montavon, and Jeger, *Helv. Chim. Acta*, 1948, **31**, 818). Their exact structure is as yet unknown, but they are especially interesting as representatives of the small group of animal triterpenes of which only two other members are known—the aliphatic squalene of shark liver oils and the tricyclic ambrein of ambergris (see below).

A chromatographic analysis of the unsaponifiable portion of wool fat led to a separation of six distinct groups, which differed strongly in their optical rotation (Daniel, Lederer, and Velluz, *Bull. Soc. Chim. biol.*, 1945, **27**, 218).

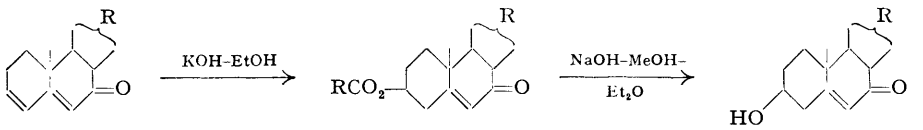
The first fraction, eluted by light petroleum, was optically inactive and consisted of a mixture of low-melting paraffins. Prelog, Ruzicka, and Stein (*Helv. Chim. Acta*, 1843, **26**, 2222) had already isolated paraffins from the unsaponifiables of pig spleen, but had classified them as "impurities," derived from the grease of the machines which had been used in the preparation of their unsaponifiables. On extracting wool in the laboratory without contact with extraneous grease, we prepared a sample of wool fat, the unsaponifiable portion of which had the same percentage of low-melting paraffins as the previously-studied samples of industrial wool fat. This shows that paraffins are genuine constituents of wool fat (and probably also of the unsaponifiables of animal organs), probably derived from the food.

The second fraction obtained by chromatography of the unsaponifiables of wool fat contained a mixture of dextrorotatory keto-steroids.

The third fraction was strongly lævorotatory, owing to the presence of cholesta-3 : 5-dien-7-one

* Unpublished results of Jutisz and Lederer; synthetic *m*-tyrosine was separated by paper chromatography from *p*-tyrosine; no natural extract showed a spot corresponding to *m*-tyrosine.

(X), $[\alpha]_D$ −350°. This substance too had already been isolated from unsaponifiables of vertebrate organs (cf. Prelog, Ruzicka, and Stein, *loc. cit.*) but it had not been possible to decide whether it was natural or an artefact. Mautner and Suida (*Monatsh.*, 1896, **17**, 579) had shown that esters (XI) of 7-ketocholesterol are hydrolysed to cholesta-3 : 5-dien-7-one (X) by alcoholic potash, but that 7-ketocholesterol (XII) is obtained by hydrolysis with ethereal sodium methoxide. We



(X.) (XI.) (XII.)

could easily decide the question as to the origin of cholesta-3 : 5-dien-7-one in wool fat by two parallel hydrolyses of wool fat; one with alcoholic potash, the other with ethereal sodium methoxide. After chromatography, only the former unsaponifiable showed the strongly lævorotatory eluates containing cholesta-3 : 5-dien-7-one. This proves that this latter substance is an artefact and that it arises from the hydrolysis of esters of 7-ketocholesterol, the presence of which is thus indicated in wool fat.

The fourth fraction obtained in the chromatography of the unsaponifiables of wool fat is again dextrorotatory and contains the afore-mentioned triterpene alcohols. The fifth fraction is lævorotatory and contains all the cholesterol. The sixth fraction, finally, is again dextrorotatory; no pure substance could be isolated from it.

Biochemistry of the triterpene alcohols of wool fat. Schulze (*Z. physiol. Chem.*, 1890, **14**, 522) had already stated that "*iso*cholesterol" gives a brown solution with a green fluorescence, in the Liebermann–Burchard reaction. Using this reaction we have developed a colorimetric method for the determination of the wool fat triterpene alcohols in presence of cholesterol. Lanosterol gives a solution with an intense absorption band at 458 mμ., whilst cholesterol and the phytosterols give a green solution with a relatively flat band at 630 mμ. (Schönheimer and Sperry, *J. Biol. Chem.*, 1934, **106**, 745). As all four triterpene alcohols of wool fat give the same colour reaction and with approximately equal intensity, it is possible to estimate the sum of triterpene alcohols by spectrophotometry at 458 mμ. (Lederer and Tchen, *Bull. Soc. Chim. biol.*, 1945, **27**, 419). This colorimetric method can be used to determine the quantity of lanolin (purified wool fat) in pharmaceutical and cosmetic preparations because lanolin contains, as we have found, an average of 11·5% of triterpene alcohols.

In studying, by this method, the "wool fat" extracted from the hair of different animals, we have found that the goat, llama, and camel also excrete small quantities of triterpene alcohols (1·2—2·5% of the "wool fat"), whereas these substances are absent from the fat from the hair of oxen, rabbits, dogs, and humans. The excretion of triterpene alcohols thus seems to be characteristic for the metabolism of certain ruminants.

We have recently examined the unsaponifiable matter of different organs of sheep, to see if the triterpene alcohols participate in the general steroid metabolism or if they are only confined to the skin and the wool (Lederer and Mercier, *Biochim. Biophys. Acta*, 1948, **2**, 91). Liver, kidneys, adrenals, pancreas, brain, pituitary, stomach, and intestine were all devoid of triterpene alcohols (their unsaponifiables containing less than 0·3% of "*iso*cholesterol"). The fat extracted from the skin, on the contrary, contains the same high percentage of triterpene alcohols as the wool fat (15—21% of the unsaponifiable). This shows that the triterpene alcohols do not participate in the steroid metabolism of the sheep, but that they are synthesized by the sebaceous glands and directly excreted on to the wool.

On examining existing evidence concerning the metabolism of the skin (*i.e.*, the sebaceous glands) we think that the peculiar composition of wool fat (branched-chain fatty acids, triterpene alcohols) is an expression of the biosynthetic capacities of the sebaceous glands, which are different from those of the liver. It would be very interesting to see whether acetate is also a precursor for the biosynthesis of the triterpene alcohols as it is for cholesterol (Block, Borek, and Rittenberg, *J. Biol. Chem.*, 1946, **162**, 441). In view of the recent paper of Srere, Chaikoff, and Dauben (*ibid.*, 1948, **176**, 829) in which the adrenal cortical tissue is established as capable also of synthesising cholesterol from acetate, it would not be astonishing if cholesterol, as well as the triterpene alcohols of wool fat, were synthesised by the sebaceous glands (see also Montagna and Noback, *Anat. Rec.*, 1946, **96**, 111).

As the scent glands of the beaver are transformed sebaceous glands, our work on castoreum stresses also the extraordinary metabolic capacities of such glands.

(4) AMBERGRIS.

Ambergris is an intestinal concretion of the sperm whale (*Physeter macrocephalus*); it forms black, brown, or grey pieces weighing sometimes several kilograms. After the death of the sperm whale, ambergris floats on the sea; under the action of air and sun its colour becomes lighter and its odour improves. Extracts of ambergris are still very much appreciated in perfumery because of their odour and fixative power.

Very little was known until recently of the constituents of ambergris. Unlike castoreum, ambergris is composed of only a few characteristic chemical components (see Table III). Let us mention them now more specifically.

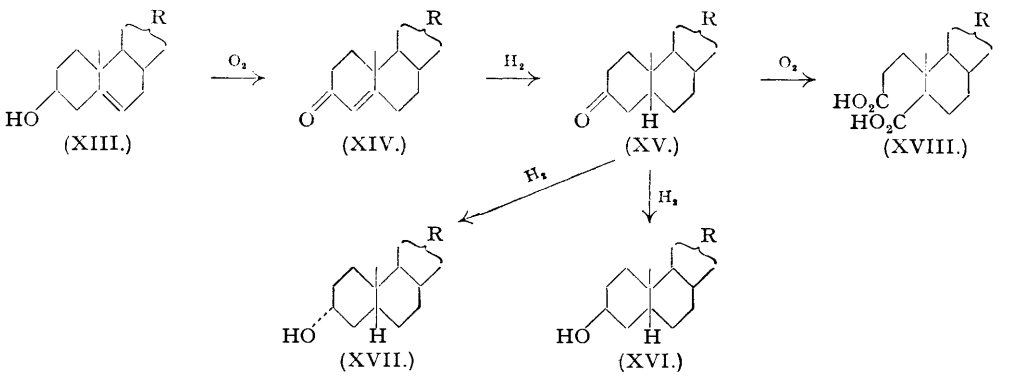
TABLE III.

Constituents of ambergris.

Ether-insoluble	10—16%	Cholesterol	0·1%
Pristane, $C_{18}H_{38}$	2— 4%	Ketones (of which more than 50% are of coprostane-3-one)	6—8%
Ambrein	25—45%		
*epi*Coprosterol, free and esterified	30—40%	Free acids	5%
Coprosterol	1— 5%	Esterified acids	5—8%

Pristane. In 1943 (*Bull. Soc. Chim. biol.*, 1943, **25**, 1237) we isolated from ambergris a liquid paraffin having the formula $C_{18}H_{28}$ (d^{20} 0·7805, n_D^{20} 1·438). The physical properties of this substance show its identity with pristane, which Tsujimoto and other Japanese authors (*J. Ind. Eng. Chem.*, 1917, **9**, 1098; *J. Soc. Chem. Ind. Japan, Suppl.*, 1937, **40**, 184B; Ueno and Komori, *ibid.*, 1935, **38**, 345B) had isolated from the liver oil of the basking shark (*Cetorhinus maximus*) and from other fish oils. We believe that the pristane of ambergris has its source in the food of the sperm whale. No details are known of the chemical constitution of this substance; as *n*-octadecane melts at 28°, pristane must have a branched structure and, as such as well as because of its animal origin, merits further study.

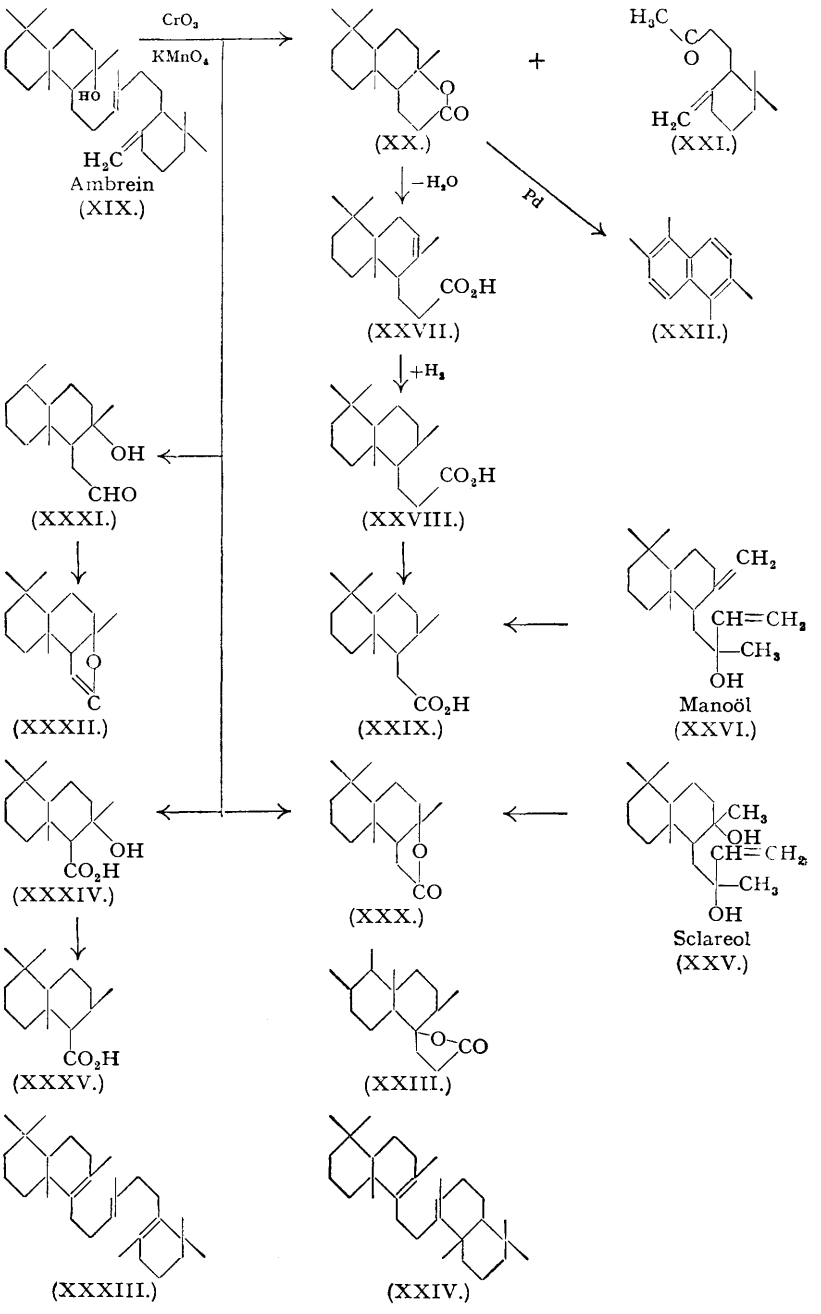
Steroids. We have confirmed the finding of Suzuki (*Jap. J. Med. Sci. Trans.*, 1925, II, **1**, 31) and of Janistyn (*Fette u. Seifen*, 1941, **48**, 501) that ambergris contains coprosterol (XVI) and



*epi*coprosterol (XVII). On treating the unsaponifiable part of ambergris with the reagent *T* of Girard and Sandulesco (*Helv. Chim. Acta*, 1936, **19**, 1095) we have isolated a crystalline keto-steroid which we identified as coprostan-3-one (XV); this is the first time this substance has been isolated from a natural source (Lederer, Marx, Mercier, and Pérot, *Helv. Chim. Acta*, 1946, **29**, 1354). Its presence in ambergris, together with coprosterol and *epi*coprosterol, is a new proof of the exactitude of the theory of Rosenheim and Starling (*Chem. and Ind.*, 1933, **48**, 238) and of Schönheimer, Rittenberg, and Graff (*J. Biol. Chem.*, 1935, **111**, 183) of the formation of coprosterol from cholesterol in the intestine of vertebrates. According to this theory, cholesterol (XIII) is first oxidised to cholest-4-en-3-one (XIV), which is reduced to coprostan-3-one (XV), which is in turn reduced to the two epimers, coprosterol (XVI) and *epi*coprosterol (XVII). Recently Rosenheim and Webster (*Biochem. J.*, 1943, **37**, 513) have isolated cholest-4-en-3-one (XIV) from the fæces of dogs and rats; and Marker, Wittbecker, Wagner, and Turner (*J. Amer. Chem. Soc.*, 1942, **64**, 818) have found *epi*coprosterol (XVII) in the fæces of dogs. Coprostan-3-one (XV) was the last intermediate of this reaction requiring isolation.

The dicarboxylic acid (XVIII), $C_{27}H_{46}O_4$, m. p. 245°, which may be prepared by chromic

acid oxidation of coprostanone was also isolated from ambergris. The recent paper of Turfitt (*Biochem. J.*, 1948, **42**, 376) on the microbial oxidation of steroids indicates that this dicarboxylic acid may be formed in the intestine of the sperm whale by microbial oxidation of coprostanone.



Ambrein. In 1820, Pelletier and Caventou (*J. Pharm.*, 1820, **6**, 49) isolated from ambergris a colourless crystalline substance which they called ambrein. Suzuki (*Jap. J. Med. Sci. Trans.*, 1925, II, **1**, 31) was the first to prepare it in a pure state (m. p. 83°). In 1946, two simultaneous publications established the chemical structure of this substance as being a tricyclic triterpene

alcohol, $C_{30}H_{52}O$, containing one tertiary hydroxyl group and two double bonds (Ruzicka and Lardon, *Helv. Chim. Acta*, 1946, **29**, 912; Lederer, Marx, Mercier, and Pérot, *loc. cit.*). Ambrein is thus the first tricyclic triterpene and a representative of the small group of animal triterpenes (see above). The study of its structure has led to results of general interest and has proceeded so rapidly that it is the first of all cyclic triterpenes whose exact chemical structure has been entirely established.

Oxidation of ambrein (XIX) by permanganate or chromic acid severs the molecule into two pieces: a saturated bicyclic lactone [ambreinolide, $C_{17}H_{28}O_2$, (XX)] and a monocyclic ketone, $C_{13}H_{22}O$ (XXI), which still contains one double bond (Lederer, Marx, Mercier, and Pérot, *loc. cit.*; Ruzicka and Lardon, *loc. cit.*). Ruzicka and Lardon (*loc. cit.*) have shown that the double bond is exocyclic—in a methylene group—and that its hydrogenation leads to tetrahydroionone. The ketone $C_{13}H_{22}O$ must thus be dihydro-γ-ionone (XXI); it has a very fine odour and has also been isolated from the volatile parts of ambergris (Ruzicka, Seidel, and Pfeiffer, *Helv. Chim. Acta*, 1948, **31**, 827). The synthesis has been accomplished by Ruzicka, Büchi, and Jeger (*ibid.*, p. 293).

The exact structure of the bicyclic part of ambrein has become known as the result of researches carried out simultaneously in Zürich and Paris.

Dehydrogenation of ambrein (XIX) or ambreinolide (XX) with selenium gives 1 : 5 : 6-trimethyl- and 1 : 6-dimethyl-naphthalene (Ruzicka and Lardon, *loc. cit.*); dehydrogenation with palladium–charcoal in our laboratory led to formation of 1 : 2 : 5 : 6-tetramethylnaphthalene (XXII). This result induced Ruzicka and Lardon to formulate ambreinolide as (XXIII), because migration of methyl groups with formation of (XXII) had as yet been observed only with hydroxylated triterpenes where it could be explained as the result of a retropinacolinic rearrangement. We showed later (Lederer, Mercier, and Pérot, *Bull. Soc. chim.*, 1947, 345) that *quadricyclo*squalene (XXIV) (Heilbron, Kamm, and Owens, *J.*, 1926, 1630; Ruzicka and Hosking, *Helv. Chim. Acta*, 1931, **14**, 203) and the plant diterpene sclareol (XXV) also give 1 : 2 : 5 : 6-tetramethylnaphthalene with our catalyst [Pd (15%) on vegetable charcoal; the usual catalyst is prepared with animal charcoal]. As a result of this finding, there was no objection to the formulæ (XX) for ambreinolide and (XIX) for ambrein.

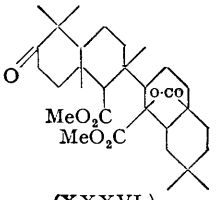
In the meanwhile, Ruzicka, Dürst, and Jeger (*Helv. Chim. Acta*, 1947, **30**, 353) had succeeded in establishing a relation between ambrein and the plant diterpene manoöl (XXVI). They had dehydrated ambreinolide (XX) to the unsaturated acid, $C_{17}H_{26}O_2$ (XXVII), which on hydrogenation gave the saturated acid, $C_{17}H_{28}O_2$ (XXVIII); on shortening the side-chain of this substance, the Swiss authors obtained an acid, $C_{16}H_{26}O_2$ (XXIX), m. p. 126°, which proved to be identical with an acid prepared by Hosking and Brandt (*Ber.*, 1935, **68**, 1311) by degradation of the plant diterpene manoöl (XXVI). This important result showed that the bicyclic parts of ambrein and manoöl are identical. As manoöl and sclareol give the same trihydrochloride (Hosking and Brandt, *Ber.*, 1935, **68**, 37), this identity includes also the bicyclic system of sclareol.

Soon afterwards, we isolated another oxidation product of ambrein, a lactone, $C_{16}H_{26}O_2$ (XXX), m. p. 121°, which proved to be identical with a lactone isolated previously by Ruzicka and Janot (*Helv. Chim. Acta*, 1931, **14**, 645) from the plant diterpene sclareol (XXV). This confirmed the identity of the bicyclic part of ambrein with that of the plant diterpenes and proved also that the hydroxyl group of ambrein was in the same position as that of sclareol. Ambreinolide was therefore a δ-lactone, and not a γ-lactone as previously supposed, and ambrein has the formula (XIX) (Lederer and Mercier, *Experientia*, 1947, **3**, 188). We had already proposed this formula in a previous paper (Lederer, Mercier, and Pérot, *loc. cit.*) after having isolated two other oxidation products of ambrein to which we had ascribed the formulæ (XXXI) and (XXXII); these two substances can also be isolated directly from ambergris.* The definitive formula of ambrein (XIX) shows a close relation to that of the (hypothetical) *tricyclo*squalene (XXXIII), as we had already pointed out in 1946 (Lederer, Marx, Mercier, and Pérot, *loc. cit.*).

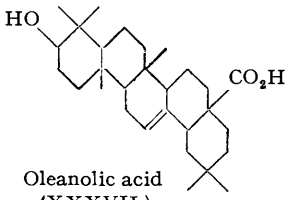
In searching for further oxidation products of ambrein, we isolated a hydroxy-acid, $C_{15}H_{26}O_3$ (XXXIV), which after dehydration and hydrogenation was transformed into the saturated acid, $C_{15}H_{26}O_2$ (XXXV), m. p. 135°. This substance proved to be identical with an acid isolated in Zürich by pyrolysis of the degradation product (XXXVI) of oleanolic acid (XXXVII), the most common pentacyclic triterpene acid derived from β-amyrin (Ruzicka, Gutmann, Jeger, and Lederer, *Helv. Chim. Acta*, 1948, **31**, 1746).

* The hydroxyl group in the formulæ for ambrein and ambreinolide had been until then placed at the carbon atom carrying the side-chain, and not on that carrying the methyl group.

As the bicylic part of ambrein had already been shown to be identical with the corresponding part of sclareol (XXV) and manoöl (XXVI) (see above), and as Jeger, Dürst, and Büchi (*Helv.*



(XXXVI.)

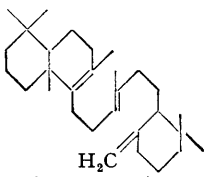


Oleanolic acid
(XXXVII.)

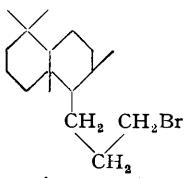
Chim. Acta, 1947, **30**, 1853) had proved the identity of the bicyclic system of the manoöl–sclareol group with that of the tricyclic diterpenes of the abietic acid group,* we thus come to the conclusion that structure and configuration of the bicyclic system of all these groups (plant diterpenes, plant triterpenes, and ambrein) is identical.

Quite recently, Meisels, Jeger, and Ruzicka (*Helv. Chim. Acta*, 1949, **32**, 1075) have isolated the same C_{15} acid from degradation products of α-amyrin.

Dürst, Jeger, and Ruzicka (*ibid.*, p. 46) have recently described a partial synthesis of ambratriene (XXXVIII), the hydrocarbon resulting from the dehydration of ambrein, by condensing the Grignard derivative of the C_{17} bromide (XXXIX), derived from ambreinolide (XIX), with dihydro-γ-ionone (XXI).



(XXXVIII.)



(XXXIX.)

We know nothing about the biogenesis of ambrein; it is a fact that the sperm whale, having teeth, has different food habits from those of other whales. Its food consists mostly of cephalopods, and one of these, *Elledone moschata*, has a scent gland and is said to have a musk-like odour. Squalene could obviously be a precursor of ambrein. We have observed no change in squalene by treating it with dilute hydrochloric acid for a week, under conditions which could prevail in the stomach of the sperm whale.

Porphyrins. Okahara (*Jap. J. Med. Sci., II., Biochem.*, 1927, **1**, 247) has described a porphyrin isolated from ambergris and called ambraporphyrin. We have shown recently that ambraporphyrin is a mixture of protoporphyrin and *meso*porphyrin which are separable by chromatography (Lederer and Tixier, *Compt. rend.*, 1947, **224**, 531). It is known that proto-porphyrin is found in the intestine of vertebrates as a result of hæmorrhages in the intestine and that it is usually partly reduced to *meso*porphyrin by the intestinal bacteria (Zeile and Rau, *Z. physiol. Chem.*, 1937, **250**, 197; Grotepass and Defalque, *ibid.*, 1938, **252**, 155). We suppose that the numerous " beaks " of cephalopods, which are always found in ambergris, provoke hæmorrhages in the intestine of the sperm whale, with subsequent formation of proto- and *meso*-porphyrins. The lesions caused by the " beaks " of the cephalopods may well be the cause of the formation of ambergris.

Perfumers will be interested to learn that probably the greater part of the odorous products of ambergris are oxidation products of ambrein and that the long-lasting smell of ambergris may be partly explained by a constant autoxidation of ambrein with formation of volatile products. This oxidation is perhaps catalyzed by the appreciable amounts of copper contained in ambergris, which are derived from the hæmocyanin of the blood of the cephalopods in the food of the sperm whale.

* This paper definitely fixed the position of the angular methyl group of ambrein.