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Degradation of Chartreusin (Antibiotic X-465A)[1]

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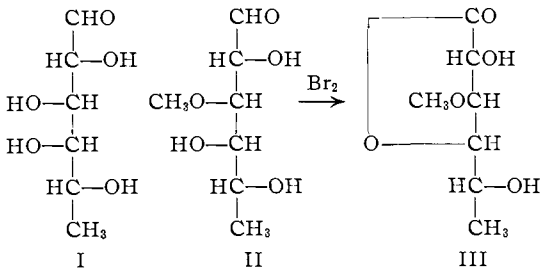
Chartreusin (antibiotic X-465A), $C_{32}H_{34-36}O_{14}$, gives on acid hydrolysis a yellow aglycone, $C_{19}H_{10-12}O_6$, and one mole each of D-fucose and D-digitalose. The aglycone is a derivative of an x-methyl-2-phenylnaphthalene or an x-methyl-2,3-benzofluorene, containing a lactone group, 2 phenolic hydroxyls and 2 oxygen atoms of undetermined nature. In the antibiotic, one of the phenolic groups is glycosidically bound to a disaccharide chain composed of the two sugars.

The preceding paper[2] describes the isolation of antibiotic X-465A from cultures of two streptomyces and its identification with chartreusin.[3] In the present paper we discuss the degradation of this antibiotic, which has given us some knowledge of its structure.

Hydrolysis of the antibiotic with dilute sulfuric acid resulted in the formation of a water-insoluble yellow aglycone, $C_{19}H_{10-12}O_6$, and of D-fucose (I) and D-digitalose (II).[4] The sugars were identified in the hydrolyzate by the preparation of D-digitalose osazone,[5] D-fucose dibenzylmercaptal[6] and D-fucose diphenylhydrazone.[7] D-Fucose and D-digitalose are formed in equimolecular amounts, since the analysis of the sugar concentrate indicated the presence of one methoxyl group per two moles of methylpentose.

A preparative separation of the sugars was achieved *via* their diethyl mercaptals. The higher melting, less soluble derivative (m.p. 166–167°), had the same m.p. as the diethyl mercaptal prepared from commercial L-fucose. Its specific optical rotation was, however, of opposite direction.[8] A mixture of the two enantiomers gave, after recrystallization, the new racemic fucose diethyl mercaptal (m.p. 158–159°).



The lower melting, more soluble diethyl mercaptal, obtained from the sugar mixture (m.p. 95–96°), analyzed correctly for the unknown D-digitalose diethyl mercaptal. It was decomposed with mercuric acetate, and the liberated sugar was oxidized to D-digitalonolactone (III). The latter was compared with an authentic sample,[9] which gave no mixed m.p. depression.

The aglycone IV, which forms yellow needles melting at 310–311° (cor.), is soluble in aqueous alkali, but is practically insoluble in most of the common organic solvents. Its elementary analysis indicates the composition $C_{19}H_{10-12}O_6$.[10] The study of a series of transformation and degradation products has shown that the aglycone IV is a derivative of an x-methyl-2-phenylnaphthalene or an x-methyl-2,3-benzofluorene, containing a lactone group, two phenolic hydroxyl groups and two additional oxygen atoms of undetermined nature.

The experimental studies leading to these conclusions are presented in the following three sections: (A) functional groups of the aglycone; (B) hydrogenation products of the aglycone; (C) carbon skeleton of the aglycone.

(A) **Functional Groups of the Aglycone** (Table I).—Titration of the aglycone IV in dimethylformamide[11] established the presence of at least one acidic group. Since the aglycone is not stable in alkali, the reaction with diazomethane was used to obtain additional proof for the presence of acidic groups. Prolonged treatment of an aglycone suspension in dimethylformamide with diazomethane resulted in the formation of an alkali-insoluble yellow derivative containing two methoxyls,[12] which indicates the presence of two acidic hydroxyls.[13] On the other hand, acylation of the yellow aglycone with acetic anhydride, or with methanesulfonyl chloride in pyridine,[14] resulted in the formation of colorless diacyl derivatives. It was not possible to acetylate the dimethyl ether, or to further methyl-

(1) Presented in part by M. W. G. at the XIVth International Congress of Pure and Applied Chemistry in Zurich (1955); Congress Handbook, p. 233. The C_{18} formulas in the summary are misprints and should read C_{19}.

(2) Julius Berger, L. H. Sternbach, R. G. Pollock, E. R. La Sala, S. Kaiser and M. W. Goldberg, THIS JOURNAL, **80**, 1636 (1958).

(3) B. E. Leach, K. M. Calhoun, L. E. Johnson, C. M. Teeters and W. G. Jackson, *ibid.*, **75**, 4011 (1953).

(4) This is, to the best of our knowledge, the first case in which D-digitalose has been found in combination with an aglycone of non-steroid nature.

(5) O. Th. Schmidt, W. Mayer and A. Distelmaier, *Liebigs Ann.*, **555**, 26 (1944).

(6) O. Th. Schmidt and E. Wernicke, *ibid.*, **556**, 179 (1944).

(7) We found for the diphenylhydrazones prepared from our D-fucose and from commercial L-fucose a melting point of 187–188° and not 199°, as reported by E. Votoček, *Chem. Zentr.*, **71**, I, 803 (1900), and A. Müther and B. Tollens, *Ber.*, **37**, 306 (1904).

(8) E. Votoček and V. Vesely, *ibid.*, **47**, 1515 (1914), give for the two enantiomers a melting point of 167–168.5°. The optical rotation is, however, not reported.

(9) We are indebted to Prof. T. Reichstein for a sample of D-digitalonolactone. ADDED IN PROOF.—A sample of the glucosidic antibiotic described by Arcamone, *et al.*,[5] was obtained by courtesy of Prof. A. Dimarco, Milan. The sample was found to be identical with antibiotic X-465A by m.p., mixed m.p. and rotation.

(10) F. Arcamone, F. Bizioli and T. Scotti, *Antibiotics and Chemotherapy*, **6**, 283 (1956), have also obtained a high melting aglycone of similar composition by acid hydrolysis of the glucosidic antibiotic produced by their *Streptomyces* sp. (no. 747).

(11) Titration with aqueous alkali causes decomposition.

(12) The aglycone itself does not contain alkoxyl groups.

(13) A small amount of an alkali-soluble product was also isolated, which analyzed for a monomethyl ether with an additional C bound methyl group. This compound has not yet been investigated.

(14) Methanesulfonyl chloride was used whenever possible for acylation, since the analytical results obtained from mesyl derivatives were more informative than the results obtained from acetyl derivatives.

ate the diacyl derivatives,[15] which indicates that the methyl and the acyl groups must be attached to the same oxygen atoms. This proves that the acidic character of the aglycone is not due to a carboxyl, but to the presence of two phenolic groups. That no structural change occurs in the acylation reactions was shown by the quantitative recovery of the aglycone upon acid hydrolysis of its diacetyl derivative. The latter is optically inactive, and this proves, therefore, also the optical inactivity of the aglycone itself. The aglycone remains unchanged on treatment with hydroxylamine in boiling pyridine, on refluxing with pyridine hydrochloride, and on boiling with a mixture of 37% hydrobromic acid and acetic acid. These observations indicate the absence of reactive carbonyl groups and readily cleavable ether groups.

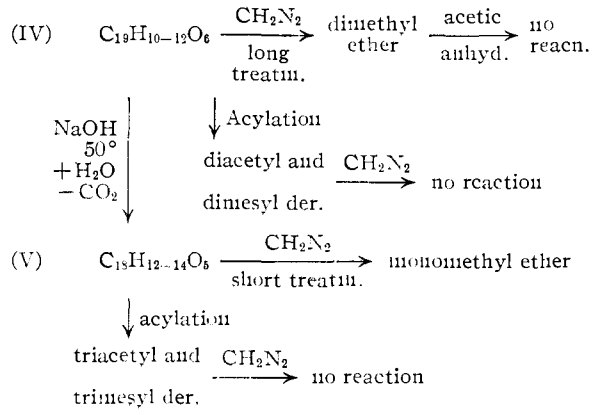
The aglycone and the antibiotic from which it is derived give a dark green color with ferric chloride in dimethylformamide solution. This color reaction is not given by the aglycone dimethyl ether. One can, therefore, conclude that the antibiotic contains a free phenolic group and that, consequently, the two sugars present in it must be glycosidically bound to the second phenolic group in form of a disaccharide chain.

The aglycone VI is rapidly decomposed in alkaline solutions at 50° to a new yellow compound, $C_{18}H_{12-14}O_5$ (V). Acylation of compound V in pyridine gave colorless triacetyl and trimesyl derivatives, which proves the presence of three hydroxyl groups.[16] Treatment of the trimesyl derivative with diazomethane resulted in the recovery of unchanged starting material, while short treatment of V itself with diazomethane gave a yellow monomethyl ether.[17] This indicates that one of the three hydroxyls present in compound V is of a strongly phenolic type and is, therefore, more readily methylated than the others. Of the two remaining hydroxyls, one is definitely also a phenol, as shown by the formation of the aglycone dimethyl ether, while the nature of the third hydroxyl is unknown. Compound V does not contain a carboxyl group. In comparison to the aglycone IV, its empirical formula contains two additional hydrogens, and one carbon and one oxygen less. Such a compound could be formed from the aglycone IV by hydrolysis of a lactone group, followed by decarboxylation of the hydroxy acid formed. Table I summarizes these reactions. The instability of certain phenolic carboxylic acids is well known and could readily explain our observations. The presence of a lactone group in the aglycone IV is also supported by the study of its hydrogenation products, discussed below.

Figure 1 contains the absorption spectra of the antibiotic, the aglycone IV, the dimethyl ether of the aglycone and its diacetyl derivative. The antibiotic and the aglycone have very similar spectra, indicating that the cleavage of the antibiotic to aglycone, D-fucose and D-digitalose is a simple hydrolysis, not accompanied by rearrangements. The shifts observed in the absorption spectra of the aglycone dimethyl ether and its diacetyl derivative are in the expected range for such compounds.

TABLE I

REACTIONS OF THE AGLYCONE



(B) **Hydrogenation Products of the Aglycone** (Table II).—Hydrogenation was used to obtain products which might lend themselves better to degradation than the aglycone itself. The hydrogenations were carried out with platinum oxide as catalyst and acetic acid as solvent, at various temperatures and pressures. Under relatively mild conditions (90° and atmospheric pressure) only one product, $C_{19}H_{14}O_5$ (VI), was formed, which crystallized in yellowish needles melting at 253–254°. Under more drastic conditions (130–145°, 70 atm. pressure), five crystalline products and a viscous oil (VI–XI, Table II) were obtained. One of the five products was $C_{19}H_{14}O_5$ (VI). In addition to that, the following compounds were isolated: $C_{19}H_{18-20}O_5$ (VII), colorless prisms, m.p. 199–204°; $C_{19}H_{16}O_4$ (VIII), yellowish needles, m.p. 254–256°; $C_{19}H_{20}O_4$ (IX), colorless needles, m.p. 203–204°; $C_{19}H_{22}O_4$ (X), dimorphic colorless prisms, m.p. 113–114° and 138–140°. The viscous oil XI, probably a mixture, analyzed for about $C_{19}H_{24-26}O_3$.

The hydrogenation products obtained in larger yields (VI, VIII and X), were submitted to the reactions used for the characterization of the aglycone *viz.*, acylation, methylation with diazomethane and treatment with alkali. The products obtained on alkali treatment were again acylated, in order to determine the number of hydroxy groups. Table II summarizes the results of these investigations and the conclusions that can be drawn from them.

The hydrogenation product VI, $C_{19}H_{14}O_5$, gives a monomethyl ether with diazomethane, and monoacyl derivatives with acetic anhydride and methanesulfonyl chloride. It still contains the lactone group present in the aglycone, since hydrolysis with NaOH leads to loss of CO_2 and formation of a new compound, $C_{18}H_{16}O_4$ (XII). As expected, the latter contains two hydroxyls, proven by the preparation of diacyl derivatives. Compound VI is thus a dihydro or tetrahydro derivative of the aglycone, in which one of the phenolic hydroxyls has

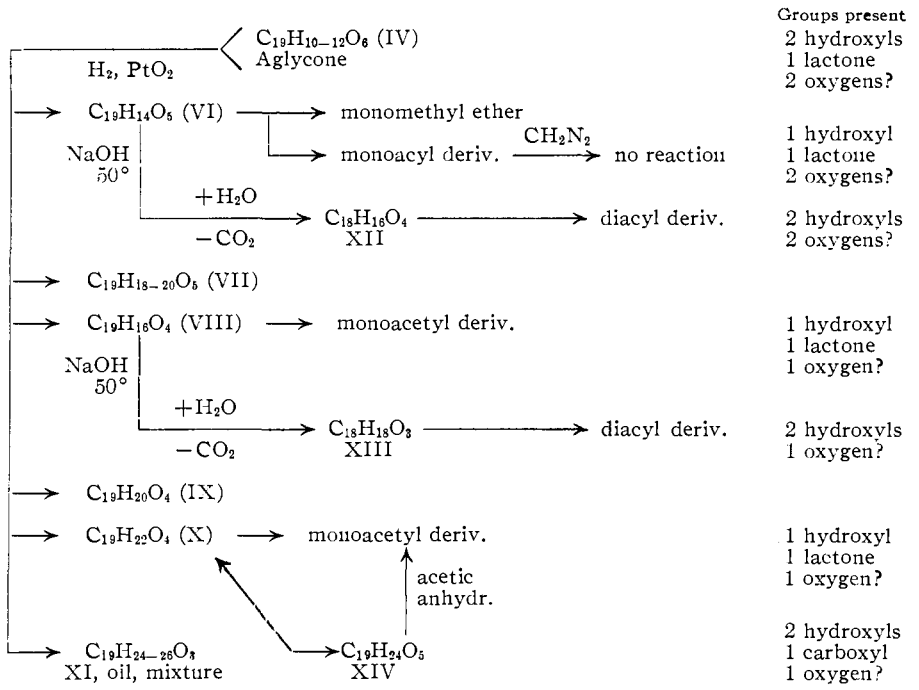
(15) On prolonged treatment with diazomethane, in the presence of water, small amounts of monomethyl-monoacyl derivatives were isolated. Similar replacements of acyl groups by methyl groups have been described by E. Baer and J. Maurukas, *J. Biol. Chem.*, **212**, 39 (1955), and J. Herzig and J. Tichatschek, *Ber.*, **39**, 1557 (1906).

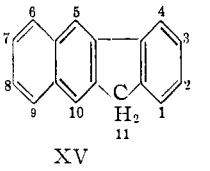
(16) Schotten-Baumann acylation with methanesulfonyl chloride gave the same trimesyl derivative in lower yield.

(17) Prolonged treatment of compound V with diazomethane gave a mixture of reaction products which has not yet been separated.

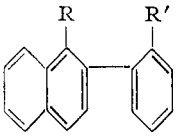
TABLE II

HYDROGENATION PRODUCTS OF THE AGLYCONE





XV



XVI

been eliminated by hydrogenolysis. The two oxygen atoms of undetermined nature are still present. Its ultraviolet spectrum (in isopropyl alcohol) shows a very high peak at 231 mμ (ε 43,400), and has additional maxima at 258 mμ (ε 22,500), 368 mμ (ε 14,200) and 388 mμ (ε 13,800).

Compound VIII, $C_{19}H_{16}O_4$, gives a monoacetyl derivative and contains, therefore, a hydroxyl. It also contains a lactone group of the same nature as in the aglycone, since NaOH hydrolysis leads to loss of CO_2 and formation of $C_{18}H_{18}O_3$ (XIII), which gives diacyl derivatives. One of the two hydroxyls and one of the two unknown oxygens, present in the aglycone, have been eliminated in compound VIII by hydrogenolysis. One oxygen atom of undetermined nature remains.

The more highly reduced compound X, $C_{19}H_{22}O_4$, gives a monoacetyl derivative, proving the presence of a hydroxyl. It also contains a lactone group. However, in this case the lactone group is not of the same nature as in the aglycone IV, and its hydrogenation products VI and VIII, since it can be hydrolyzed with NaOH to the corresponding hydroxy acid, $C_{19}H_{24}O_5$ (XIV), without loss of CO_2. Treatment with acetic anhydride converts the hydroxy acid XIV to the monoacetyl derivative of lactone X, and heating to 130° gives starting material, the free hydroxy lactone X. In the latter, one of the two hydroxyls and one of the two oxygens of unknown nature, originally present in the aglycone IV, are eliminated. One oxygen atom of unknown nature remains. The ultraviolet absorption spectrum of compound X reflects its more saturated nature. It shows (in 95% ethanol) maxima at 236 mμ (ε 12,700), 267 mμ (ε 8,400) and 352 mμ (ε 5,600).

Compounds VII and IX (Table II) were obtained in minute amounts only and have, therefore, not yet been investigated. The more readily avail-

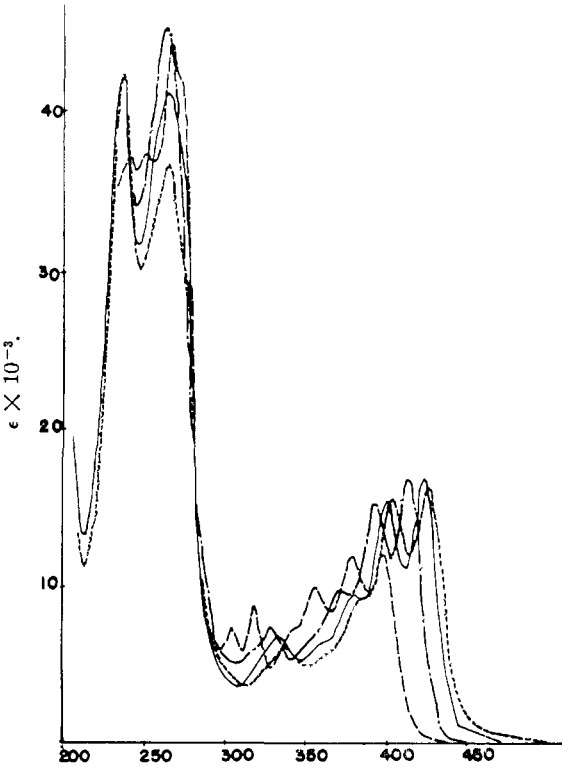


Fig. 1.—Ultraviolet absorption spectra: antibiotic (95% ethanol), ———; aglycone (95% ethanol), ·····; aglycone dimethyl ether (isopropyl alcohol + 2% dimethylformamide), — · —; aglycone diacetyl derivative (ethanol + 2% dioxane), — — —.

able hydrogenation products will be used for further degradation studies.

(C) **Carbon Skeleton of the Aglycone.**—Palladium–charcoal dehydrogenation of the more highly reduced aglycone derivatives was used to determine the carbon skeleton, since zinc dust distillation of the aglycone had not given useful results. The highly reduced aglycone derivatives contain less oxygen than the aglycone, and we thought that this should facilitate their dehydrogenation to the aromatic hydrocarbon, representing the carbon skeleton. The dehydrogenation reactions were carried out with the oil (XI, Table II), analyzing for about $C_{19}H_{24-26}O_3$, and also with the crystalline reduction product $C_{19}H_{22}O_4$ (X). In both cases, there were obtained the same two crystalline 2,4,7-trinitrofluorenone (TNF) derivatives (m.p. 219–220° and 142–143°) of two hydrocarbons, in addition to a considerable amount of material not reacting with TNF.

The analyses of the higher melting TNF derivative and of the hydrocarbon obtained from it (m.p. 222–222.5°) indicated for the hydrocarbon an empirical formula of $C_{18}H_{14}$. The hydrocarbon contains thus one carbon atom less than the aglycone. This missing carbon atom is probably that of the carbonyl group of the lactone ring, lost in the dehydrogenation process. The other TNF derivative (m.p. 142–143°) yielded a liquid hydrocarbon. The analyses of the TNF derivative and of the 1,3,5-trinitrobenzene (TNB) derivative of the hydrocarbon (containing 2 moles TNB per mole hydrocarbon) indicated for the hydrocarbon the composition $C_{17}H_{14}$, which is two carbon atoms less than present in the starting material.

Since these hydrocarbons could not be identified with compounds described in the literature, their ultraviolet absorption spectra were compared with the spectra of known hydrocarbons of similar composition.

As shown in Fig. 2, the ultraviolet absorption spectrum of the crystalline hydrocarbon, $C_{18}H_{14}$, is practically identical with that of 2,3-benzofluorene,

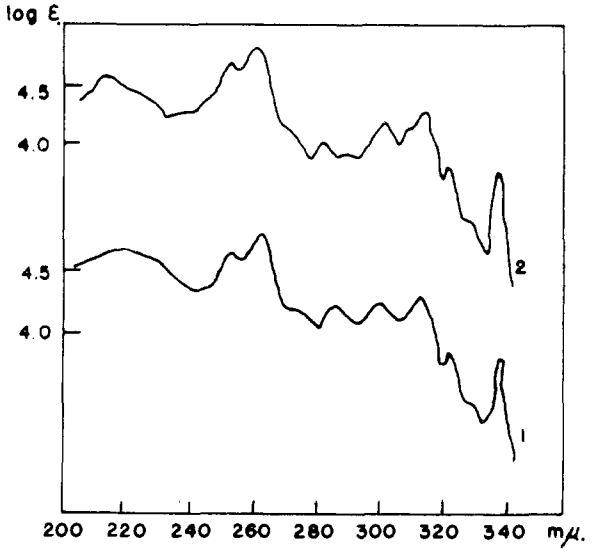


Fig. 2.—Ultraviolet absorption spectra of x-methyl-2,3-benzofluorene (1) and 2,3-benzofluorene (2).

$C_{17}H_{12}$ (XV).[18] The physical properties of 2,3-benzofluorene (m.p. 212–213°) and its TNF derivative (221–222°), as described in the literature, closely resemble those of the new hydrocarbon and its TNF derivative. However, a direct comparison of our hydrocarbon and its TNF derivative with purified samples of commerical 2,3-benzofluorene and its TNF derivative, revealed characteristic differences. The most likely conclusion is that the new hydrocarbon is a methyl derivative of 2,3-benzofluorene. This methyl derivative could be any one of the 11 possible isomers, none of which is described in the literature.

The second hydrocarbon, regenerated as a liquid from its chemically pure TNF derivative (m.p. 142–143°), gave an ultraviolet absorption spectrum (Fig. 3) which is almost identical with that

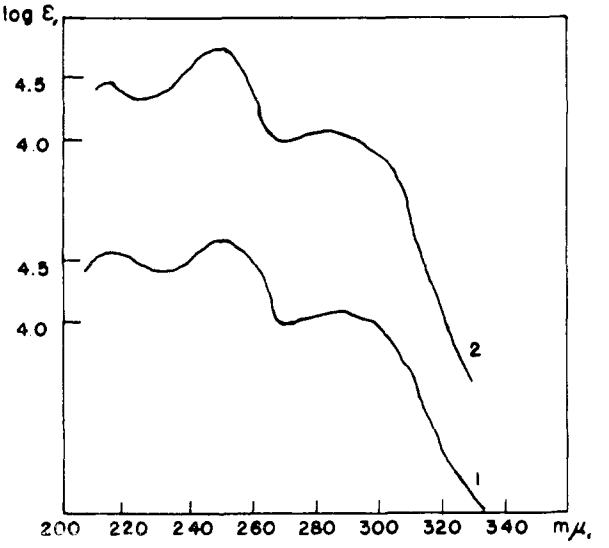


Fig. 3.—Ultraviolet absorption spectra of x-methyl-2-phenylnaphthalene (1) and 2-phenylnaphthalene (2).

of 2-phenylnaphthalene, $C_{16}H_{12}$ (XVI, R, R′ = H).[19] However, the latter is a crystalline compound melting at 102–103°. It appears, therefore, that our liquid hydrocarbon is a methyl-2-phenylnaphthalene. This assumption is supported by the fact that this hydrocarbon, like 2-phenylnaphthalene and 2-(*o*-tolyl)-naphthalene, forms a complex with two moles of *sym*-trinitrobenzene per mole of hydrocarbon.[18] The simultaneous formation of a methyl-2-phenylnaphthalene and of a methyl-2,3-benzofluorene from the same material can be explained if one assumes the loss of the (possibly oxygen-substituted) methylene group 11 of the 2,3-benzofluorene derivative undergoing dehydrogenation.[20]

Only two methyl-2-phenylnaphthalenes are known, the 1-methyl derivative[21] (XVI, R = CH_3, R′ = H), melting at 84°, and the 2-(*o*-tolyl)-naphthalene (XVI, R = H, R′ = CH_3), melting

(18) M. Orchin and R. A. Friedel, THIS JOURNAL, **71**, 3002 (1949).

(19) R. A. Friedel, M. Orchin and L. Reggel, *ibid.*, **70**, 199 (1948).

(20) Formation of the five-membered ring by dehydrocyclization is less likely. For example, in the synthesis of 2-(*o*-tolyl)-naphthalene (XVI, R = H, R′ = CH_3), carried out by Friedel, *et al.*,[19] which requires a dehydrogenation step with Pd-on-carbon, formation of a benzofluorene was not observed.

(21) F. S. Spring, *J. Chem. Soc.*, 1332 (1934).

TABLE III

INFRARED ABSORPTION BANDS IN THE REGION OF 690–900 CM.$^{-1}$

w = weak band, sh = shoulder; bands not marked are strong or medium strong

2,3-Benzofluorene		868					769		760	742	723	
x-Methyl-2,3-benzofluorene		867				784				744	726	
2-Phenylnaphthalene	890		855	818			771		759	739		696
2-*o*-Tolylnaphthalene	895w		856	820	790w		770w		757	743	723	
x-Methyl-2-phenylnaphthalene	888 (sh 878)		855	818	805w	783		765w		743		699

at 45.7–48°.[19] The physical properties of both compounds differ from those of our liquid hydrocarbon. In addition, the 2-(*o*-tolyl)-naphthalene does not give a trinitrofluorenone derivative and shows a different ultraviolet absorption spectrum.[19,22]

If we assume that the two hydrocarbons obtained by dehydrogenation of the reduced aglycone derivatives are related as outlined above, then the methyl group in both, the x-methyl-2,3-benzofluorene and the x-methyl-2-phenylnaphthalene, should occupy analogous positions. Therefore, the x-methyl-2,3-benzofluorene could not have the methyl group in position 4 or 5. Position 11 also is excluded since, in that case, loss of carbon 11 during dehydrogenation would actually mean loss of two carbon atoms and formation of 2-phenylnaphthalene, which was not found. This leaves for the methyl group of x-methyl-2,3-benzofluorene positions 1, 2, 3, 6, 7, 8, 9 and 10.

Additional information was obtained by comparing the infrared spectra of the two dehydrogenation products (in carbon disulfide solution) with the infrared spectra of 2,3-benzofluorene,[23] 2-phenyl- and 2-(*o*-tolyl)-naphthalene.[24] A number of strong bands in the 690–900 cm.$^{-1}$ region, caused by the out-of-plane deformation vibrations of the aromatic hydrogen atoms, seem to be characteristic for these five hydrocarbons (see Table III). Some occur only in the benzofluorenes, others only in the phenylnaphthalenes, and others again are common only to the x-methyl derivatives (784 cm.$^{-1}$ in the x-methyl-2,3-benzofluorene and 783 cm.$^{-1}$ in the x-methyl-2-phenylnaphthalene). They confirm the relationship of these hydrocarbons and show characteristic differences between the x-methyl and the unsubstituted compounds.

One strong absorption band in the 700 cm.$^{-1}$ region is rather suggestive. It occurs only in 2-phenylnaphthalene (696 cm.$^{-1}$) and in the x-methyl-2-phenylnaphthalene (699 cm.$^{-1}$), and does not occur in *o*-tolylnaphthalene or in the benzofluorenes. It is due possibly to the presence of five hydrogen atoms in the phenyl ring, since a band at 700 ± 10 cm.$^{-1}$ is being assumed to be characteristic for a monosubstituted phenyl group.[25] If this interpretation is correct, it would exclude the presence of the methyl group in the phenyl ring, and, therefore, of a methyl group in positions 1, 2, 3 and 4 of the 2,3-benzofluorene molecule. The methyl group could thus only be in 6, 7, 8, 9 or 10, since position 5 of the 2,3-benzofluorene molecule has been excluded, because of the non-identity of 1-methyl-2-phenylnaphthalene with our x-methyl-2-methyl-2-phenylnaphthalene. The final proof of structure of our hydrocarbons requires the synthesis of some or of all of the above indicated isomers.

In the aglycone, $C_{19}H_{10-12}O_6$, which must be a derivative of an x-methyl-2-phenylnaphthalene or an x-methyl-2,3-benzofluorene, the methyl group also could be substituted. For example, it could be a methylene group in an ether. Its migration from an angular position during the dehydrogenation process, while not likely in this case ($C_{19}H_{10}O_6$ corresponds to an aromatic structure), also can not be excluded completely.

Probable Structure of Chartreusin (Antibiotic X-465A).—The above discussed experimental findings lead to a tentative structure for this antibiotic: It is a derivative of 2-phenylnaphthalene or of 2,3-benzofluorene, containing a lactone group, 2 phenolic hydroxyls and 2 oxygen atoms of undetermined nature. There is an additional methyl or a methylene group present, and one of the phenolic groups is glycosidically bound to a disaccharide chain, composed of D-fucose and D-digitalose.

Acknowledgments.—We wish to thank Dr. Al Steyermark for the microanalyses, Dr. A. Motchane and his staff for the infrared and ultraviolet absorption spectra and Mr. B. Pecherer for some of the infrared data.

Experimental[26]

WITH THE ASSISTANCE OF E. REEDER

Hydrolysis of the Antibiotic.—To a solution of 10 g. of antibiotic in 1200 cc. of (peroxide free) dioxane was added 190 cc. of 3 *N* sulfuric acid.[27] The mixture was stirred and refluxed for 45 minutes. It was then concentrated *in vacuo* to 600 cc., diluted with 200 cc. of water and cooled for 15 hours to +5°. The aglycone precipitated in form of yellow needles (5.12 g.) and was filtered off. The dilute dioxane solution was freed from sulfuric acid ions with barium hydroxide, treated with charcoal and concentrated *in vacuo*, yielding 5.5 g. of an almost colorless, sweet tasting sirup. The analysis showed the presence of 9.2% methoxyl. The calculated value for an equimolar mixture of fucose and digitalose is 9.1%.

D-Digitalose Osazone.—A solution of 0.42 g. of the sugar sirup, 1.2 g. of phenylhydrazine hydrochloride and 1.2 g. of crystalline sodium acetate in 8 cc. of water containing 6 drops of glacial acetic acid was heated on the steam-bath for 5 hours. After cooling, the brownish-yellow precipitate was filtered off and recrystallized repeatedly from 60% methanol. Yellow needles melting at 177–178° were finally obtained.

Anal. Calcd. for $C_{19}H_{24}O_3N_4$: C, 64.02; H, 6.80; N, 15.72; OCH_3, 8.70. Found: C, 63.99, 63.98; H, 6.63, 6.65; N, 16.22; OCH_3, 8.93.

D-Fucose Diphenylhydrazone.—To a solution of 1 g. of the sugar sirup and 1.55 g. of diphenylhydrazine hydro-

(22) The ultraviolet absorption spectrum of 1-methyl-2-phenylnaphthalene has not been published.

(23) Commercial material (Aldrich Chemical Co.) was purified *via* its picrate and TNF derivative.

(24) Dr. Leslie Reggel, U. S. Bureau of Mines, Pittsburgh, Pa., kindly furnished the samples of 2-phenyl and 2-(*o*-tolyl)-naphthalene.

(25) See L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen and Co., Ltd., London, 1954, p. 65.

(26) All melting points are corrected.

(27) The hydrolysis was also carried out with hydrochloric acid.

chloride in a few cc. of water were added 6.5 cc. of 1 *N* sodium hydroxide and sufficient alcohol to obtain a clear solution. After neutralizing with a few drops of pyridine, the mixture was refluxed for 10 minutes. It was then partly concentrated *in vacuo*, and the residue dissolved by heating with dilute alcohol. Cooling caused the formation of a partly crystalline precipitate, which was filtered off and washed with water and ether (0.43 g., m.p. 183–186°). Crystallization from alcohol gave fine white needles melting at 187–188°.

Anal. Calcd. for $C_{18}H_{22}O_4N_2$: C, 65.44; H, 6.71; CH_3O, 0. Found. C, 65.29; H, 7.02; CH_3O, 0.

D-Fucose Dibenzylmercaptal.—To a solution of 0.8 g. of the sugar sirup in 1.5 cc. of concentrated hydrochloric acid was added 2.5 cc. of benzylmercaptan. The mixture was heated to 50° and shaken for 20 minutes. It was then diluted with water, the crystals that formed were filtered off, washed with water and ether, and recrystallized from methanol (0.25 g.); long needles melting at 181–183°, $[\alpha]^{24}D$ −26.3 ± 0.5° (*c* 0.9% in pyridine[28]).

Anal. Calcd. for $C_{20}H_{26}O_4S_2$: C, 60.88; H, 6.64. Found: C, 61.22; H, 7.04.

Diethylmercaptals of D-Fucose and D-Digitalose.—A solution of 6 g. of the sugar sirup in 9 cc. of concd. hydrochloric acid was shaken at room temperature with 9 cc. of ethyl mercaptan. After a few minutes a heavy precipitate was formed, which was filtered off after adding some water. The mother liquors were concentrated *in vacuo* to 10 cc. and saturated in the cold with hydrogen chloride. Ethyl mercaptan (10 cc.) was again added and the mixture shaken at room temperature for 1 hour. The mixture was again diluted with water and filtered, yielding another crop of crystals. Both fractions of crystals were dried, combined and extracted with ether. The undissolved part, the D-fucose derivative, had a melting point of 164–165° and weighed 3.0 g. which corresponds to a yield of 64%. For the analysis, a sample was recrystallized from alcohol; long colorless needles melting at 166–167°, $[\alpha]^{24}D$ +9.5 ± 1° (*c* 1 in pyridine).[29]

Anal. Calcd. for $C_{10}H_{22}O_4S_2$: C, 44.44; H, 8.20. Found: C, 44.21; H, 8.28.

The ether solution, containing the soluble D-digitalose diethylmercaptal, was concentrated *in vacuo*, and the residue recrystallized from a mixture of ether and petroleum ether. The product crystallized in fine needles melting at 95–96°, $[\alpha]^{24}D$ −11.5 ± 0.5° (in pyridine, *c* 2). The yield was 1.03 g., 21%.

Anal. Calcd. for $C_{11}H_{24}O_4S_2$: C, 46.47; H, 8.51. Found: C, 46.58, 46.62; H, 8.79, 8.61.

Racemic Fucose Diethylmercaptal.—A mixture of equal parts of D- and of L-fucose diethylmercaptal was recrystallized from alcohol. The racemate crystallized in heavy plates melting at 158–159°.

Anal. Calcd. for $C_{10}H_{22}O_4S_2$: C, 44.44; H, 8.20. Found: C, 44.63; H, 7.85

D-Digitalonolactone from D-Digitalose Diethylmercaptal.—To a solution of 1.2 g. of D-digitalose diethylmercaptal (m.p. 95–96°) in 40 cc. of 65% alcohol was added a solution of 2.4 g. of mercuric acetate in 15 cc. of methanol. The mixture was refluxed for 35 minutes, diluted with water, filtered, treated with hydrogen sulfide and filtered again after addition of some activated carbon and Hyflo. The solution was concentrated *in vacuo*, giving 0.52 g. of crude sirupy digitalose, which was dissolved in 10 cc. of water and oxidized as follows: Bromine (0.5 cc.) was added, and the mixture was shaken for 10 minutes, then left at room temperature in the dark for 24 hours. The bromine was removed *in vacuo*, the residual solution diluted with water, treated with silver carbonate, filtered, treated with hydrogen sulfide and filtered again after addition of some activated carbon and Hyflo. The solution was then concentrated *in vacuo* and the residue (0.5 g.) which became crystalline after a few hours, was distilled at 0.1 mm. in a "bulb tube" at an air bath temperature of 140–160°. The partly crystalline distillate (0.36 g.) was crystallized from a mixture of acetone and petroleum ether, yielding 0.2 g. of a crystalline product melting at 132–134°. After two recrystallizations from a mixture of acetone and petroleum ether, 110 mg. of needles was obtained melting at 136–137°.[30] The product gave no melting point depression with an original sample of D-digitalonolactone obtained from Prof. T. Reichstein. The specific optical rotation of two samples was $[\alpha]^{28}D$ −77.5 ± 1.5° and $[\alpha]^{25}D$ −82 ± 2° (*c* 1, in water). The rotation was determined about 15 minutes after preparing the solution. There was no change after 27 hours.[31]

Aglycone.—The yellow needles (m.p. 310–311°) which were filtered off after hydrolysis of the antibiotic represented chemically pure aglycone. The same product also can be obtained by thermal decomposition of the antibiotic at 250° and 0.2 mm. pressure. The aglycone sublimes out under these conditions. Samples for the analysis were recrystallized from large amounts of methylene chloride, from boiling dioxane (0.16 g. in 100 cc.), or from hot dimethylformamide (0.2 g. in 20 cc.), and showed no change in melting point or appearance. The aglycone forms a yellow solution in concentrated sulfuric acid. The orange colored solution in alkali turns a deep purple on heating in the presence of air.

Anal. Calcd. for $C_{19}H_{10}O_6$: C, 68.27; H, 3.02; mol. wt., 334.27. Calcd. for $C_{19}H_{12}O_6$: C, 67.85; H, 3.60; mol. wt., 336.29. Found: C, 68.06, 68.35, 68.18; H, 3.35, 3.26, 2.96; OCH_3, 0; acetyl, 0; equiv. wt., 353 (titr. in dimethylformamide).

Diacetyl Derivative of the Aglycone.—To a solution of 0.5 g. of the aglycone in 150 cc. of dry warm pyridine were added 30 cc. of acetic anhydride. The mixture was left at room temperature for 14 hours and was then concentrated *in vacuo* to a small volume. The slightly pink crystals (0.6 g.) were filtered off and recrystallized from chloroform with the addition of activated carbon. The product forms white needles melting at 292–294°. It is optically inactive (*c* 0.3 in pyridine).

Anal. Calcd. for $C_{23}H_{14}O_8$: C, 66.02; H, 3.37; acetyl, 20.8. Calcd. for $C_{23}H_{16}O_8$: C, 65.71; H, 3.84; acetyl, 20.7. Found: C, 65.86, 65.70, 66.18; H, 3.55, 3.75, 3.75; acetyl, 19.18, 18.96.[32]

Dimesyl Derivative of the Aglycone.—A solution of 0.5 g. of aglycone in 190 cc. of warm dry pyridine was cooled quickly and treated with 2 cc. of methanesulfonyl chloride. The mixture was left for 24 hours at room temperature. It was then diluted with water and partly concentrated *in vacuo*. The precipitated crystals were filtered off, washed with water and recrystallized from dimethylformamide. The product forms white needles melting at 279–281°. The yield was practically quantitative.

Anal. Calcd. for $C_{21}H_{14}O_{10}S_2$: C, 51.44; H, 2.88. Found: C, 51.48; H, 2.67; MeO, 0.

Monomethyl Monoacetyl Aglycone.—To a solution of 0.4 g. of diacetyl aglycone in 240 cc. of dimethylformamide containing some water was added an excess of an ether solution of diazomethane. The mixture was left at room temperature for 20 hours, and was then concentrated *in vacuo* to a small volume. The precipitated unreacted material was filtered off. The rest of the solution was concentrated to dryness, and the residue (50 mg.) was purified by crystallization from a mixture of chloroform and petroleum ether, and finally from acetone. The product forms yellow needles melting at 274–276°, and gives a melting point depression with the diacetyl aglycone. It produces an orange-yellow color with concentrated sulfuric acid.

Anal. Calcd. for $C_{22}H_{14}O_7$: C, 67.69; H, 3.62; MeO, 7.95. Found: C, 67.78, 68.17; H, 3.78, 3.57; MeO, 5.90.

Monomethyl Monomesyl Aglycone.—To a solution of 80 mg. of dimesyl aglycone in 50 cc. of dimethylformamide containing some water was added an excess of diazomethane in ether. The mixture was left at room temperature for 14 hours, and was then concentrated *in vacuo*. The soluble

(28) O. Th. Schmidt and E. Wernicke, *Liebigs Ann.*, **556**, 179 (1944), report a melting point of 184° and $[\alpha]^{20}D$ of −27.8° (*c* 2.8 in pyridine).

(29) The product gave no melting point depression with the diethylmercaptal prepared from D-fucose. We are indebted to Prof. T. Reichstein for the D-fucose sample.

(30) A. Rheiner, A. Hunger and T. Reichstein, *Helv. Chim. Acta*, **35**, 687, 712 (1952), report a melting point of 138–139°.

(31) The specific optical rotation reported in the literature varies between −92.5° and −75.9 ± 3°. See O. Schindler and T. Reichstein, *ibid.*, **35**, 442 (1952).

(32) Acetyl and methoxyl determinations in this series of compounds (aglycone and its hydrogenation products) gave considerable difficulties. In most cases low values were obtained.

part was extracted with hot acetone and crystallized from acetone. The product forms yellow needles (about 15 mg.) melting with decomposition at 271–274°.

Anal. Calcd. for $C_{21}H_{14}O_8S$: C, 59.16; H, 3.31; MeO, 7.2. Calcd. for $C_{21}H_{16}O_8S$: C, 58.88: H, 3.77; MeO, 7.2. Found: C, 58.90; H, 3.96; MeO, 5.75.

Methylation of Aglycone.—A solution of 5 g. of aglycone in 1600 cc. of hot dimethylformamide was cooled and treated for one hour at room temperature with a large excess of diazomethane in ether. The solution was then concentrated *in vacuo* and the residue was crystallized from methylene chloride. Some impure unreacted aglycone (0.47 g.) precipitated first and was filtered off. After partial concentration, various unsharp melting fractions (240–260°) were obtained and were purified chromatographically as described below. The mother liquors were concentrated and the residue chromatographed separately.

A solution of 2.1 g. of the combined crystalline fractions in 225 cc. of methylene chloride was poured on a column containing 200 g. of acetic acid washed alumina. The column was eluted with methylene chloride (1000 cc.) and yielded 1.2 g. of yellow prisms (A). Further washing with methylene chloride and ethyl acetate gave only small amounts of impure material. The column was then extruded and divided into several parts differing in color. All parts were extracted with boiling ethanol. The lowest, greenish-yellow layer yielded 0.2 g. of brownish-yellow needles (B), the other layers yielded only very small amounts of unidentified products. The above-mentioned mother liquors yielded after chromatographic purification 0.8 g. of the yellow prisms (A).

Aglycone Dimethyl Ether.—The yellow prisms (A) obtained from both columns were purified by crystallization from methylene chloride and yielded 1.9 g. of yellow prisms melting at 282–283°. They are insoluble in alkali. The color in concentrated sulfuric acid is orange, while the aglycone forms a yellow solution.

Anal. Calcd. for $C_{21}H_{14}O_6$: C, 69.61; H, 3.89; MeO, 17.1. Calcd. for $C_{21}H_{16}O_6$: C, 69.22; H, 4.43; MeO, 17.1. Found: C, 69.30, 69.23; H, 3.89, 4.06; MeO, 15.66, 15.61.

Acetylation of the compound was attempted by heating for 1 hour at 90° with a mixture of pyridine and acetic anhydride. Unchanged aglycone dimethyl ether was recovered.

Anal. Found: C, 69.47; H, 3.80; acetyl, 0.

Product B.—The substance (0.2 g.) was purified by crystallization from methylene chloride. It forms yellow needles melting at 284–286° and gives a melting point depression with the aglycone dimethyl ether (A). The analyses check for a C-methyl O-methyl aglycone derivative, rather than for a monomethyl ether.

Anal. Calcd. for $C_{20}H_{12}O_6$: C, 68.96; H, 3.47; 1 MeO, 8.9. Calcd. for $C_{21}H_{14}O_6$: C, 69.61; H, 3.89; 1 MeO, 8.56. Found: C, 69.47, 69.70, 69.94; H, 4.08, 3.79, 4.04; MeO, 8.19, 8.81.

Alkaline Hydrolysis of the Aglycone; Formation of $C_{18}H_{12-14}O_5$ (V).—A stirred suspension of 5 g. of aglycone in a mixture of 500 cc. of methanol and 1250 cc. of 1.5 *N* sodium hydroxide was heated in an atmosphere of nitrogen. After 35 minutes the temperature reached 52° and the product was completely dissolved. The brown solution was then filtered over Hyflo into 700 cc. of 3 *N* HCl. (Air was excluded as much as possible, since an intense purple color develops on air oxidation of the alkaline solution). The acidified turbid yellow filtrate was partly concentrated *in vacuo*, heated for 2 hours to 80–90° to achieve precipitation, cooled and filtered. The amorphous yellow precipitate (3.75 g.) was washed with water and ether. It can be recrystallized from a mixture of pyridine and petroleum ether, or from diethylformamide with the addition of ether. It forms yellow prisms or needles, which char on heating and do not melt till 330°. The color in concentrated sulfuric acid is orange-brown.

Anal. Calcd. for $C_{18}H_{12}O_5$: C, 70.13; H, 3.92. Calcd. for $C_{18}H_{14}O_5$: C, 69.67; H, 4.55. Found: C, 70.13, 69.60; H, 4.47, 4.40; MeO, 0.

Triacetyl Derivative of $C_{18}H_{12-14}O_5$ (V).—Ten cc. of acetic anhydride was added to a solution of 0.2 g. of V in 10 cc. of dry pyridine. The acetylation product crystallized out in quantitative yield, and was filtered off after 4 hours. It was recrystallized from a mixture of methylene chloride and ether, forming fine needles or short prisms melting at 280–281°.

Anal. Calcd. for $C_{24}H_{16}O_8$: C, 66.67; H, 3.73; CH_3CO, 29.7. Calcd. for $C_{24}H_{18}O_8$: C, 66.36; H, 4.18; CH_3CO, 29.7. Found: C, 66.31, 66.39, 66.56; H, 4.33, 4.50, 3.95; CH_3CO, 25.61, 25.61.

The triacetyl derivative remained unchanged after treatment for one hour in dioxane solution with an excess of diazomethane. Treatment of the product in pyridine with a large excess of hydroxylamine at room temperature for 18 hours resulted in a red oil which could not be crystallized.

Trimesyl Derivative of $C_{18}H_{12-14}O_5$ (V).—To a solution of 0.5 g. of V in 20 cc. of pyridine was added 2 cc. of methanesulfonyl chloride. The mixture was left at room temperature for 2 hours and then was treated with water. The precipitated reaction product, isolated in almost quantitative yield, was filtered off, and recrystallized from a mixture of dimethylformamide and ether. The product forms white prisms melting at 279–280°.

Anal. Calcd. for $C_{21}H_{18}O_{11}S_3$: C, 46.49; H, 3.34. Calcd. for $C_{21}H_{20}O_{11}S_3$: C, 46.32; H, 3.70. Found: C, 46.23, 46.33; H, 3.29, 3.73.

A Schotten–Baumann reaction of V with methanesulfonyl chloride in alkali resulted in the formation of the same product with lower yield. The product remained unchanged on treatment in dimethylformamide with an excess of diazomethane.

Monomethyl Ether of $C_{18}H_{12-14}O_5$ (V).—To a solution of 0.2 g. of V in 50 cc. of dimethylformamide was added an excess of diazomethane in ether. The solution was concentrated *in vacuo* after a few minutes. The residue was crystallized from a mixture of acetone and petroleum ether, forming yellow needles melting at 263–266°.

Anal. Calcd. for $C_{19}H_{14}O_5$: C, 70.80; H, 4.38; MeO, 9.5. Calcd. for $C_{19}H_{16}O_5$: C, 70.36; H, 4.95; MeO, 9.5. Found: C, 70.78, 71.07; H, 4.81, 4.54; MeO, 9.21.

Catalytic Hydrogenation of the Aglycone (see Table II).—Eight grams of aglycone was hydrogenated in 1 liter of acetic acid at 130–145° and 1000 p.s.i. pressure with 3 g. of platinum oxide as catalyst. After 20 hours the mixture was cooled and removed from the autoclave, heated to redissolve the precipitated hydrogenation products, filtered and concentrated *in vacuo* to a small volume. Fine cream colored needles precipitated and were filtered off (2.5 g.). The product melted at 230–236° and was separated into two compounds by repeated fractional crystallization from pyridine (methylene chloride or dimethylformamide yielded only mixtures). Both compounds crystallized in fine yellowish needles, one melting at 254–256° (somewhat less soluble in pyridine), the other at 253–254°. They have the same physical appearance and give the same orange-yellow color reaction with concentrated sulfuric acid. The only practical way of distinguishing them is the melting point depression observed with mixtures. The lower melting product (253–254°) was finally recrystallized for analysis from a mixture of chloroform and petroleum ether, and had the composition $C_{19}H_{14}O_5$ (VI). The same product is obtained in almost quantitative yield by hydrogenation of the aglycone in acetic acid at 90° and atmospheric pressure in the presence of an equal weight of platinum oxide.

Anal. Calcd. for $C_{19}H_{14}O_5$: C, 70.80; H, 4.38. Found: C, 70.89, 71.14; H, 4.47, 4.59.

The other compound had lost one oxygen atom and analyzed for $C_{19}H_{16}O_4$ (VIII).

Anal. Calcd. for $C_{19}H_{16}O_4$: C, 74.01; H, 5.23. Found: C, 73.88, 73.71; H, 5.16, 4.95.

The filtrate, after removal of the needles melting at 230–236°, was diluted stepwise with petroleum ether and yielded inorganic material (0.26 g.), introduced with the platinum catalyst, then fine cream colored needles, 0.18 g. melting at 203–204° (IX). The melting point did not change after two recrystallizations from ethanol. The color in concd. sulfuric acid is yellow.

Anal. Calcd. for $C_{19}H_{20}O_4$: C, 73.06; H, 6.45. Found: C, 72.80, 72.94; H, 6.31, 6.65; CH_3CO, 0.

The residual solution was concentrated *in vacuo* yielding 4.5 g. of oil, which crystallized partly after some time. It was triturated with a mixture of ether and petroleum ether,

yielding finally 3 g. of oil and 1.1 g. of crystals. The solid material (1.1 g.) crystallizes from a mixture of ether and petroleum ether in two forms, depending on the ratio of solvents. It forms well-developed prisms melting at 140–141° or irregular prisms melting at 116–117° (X). Both forms can be converted into each other by crystallization. Both are colorless. The color in concentrated sulfuric acid is yellow. The product shows no optical rotation in pyridine.

Anal. Calcd. for $C_{19}H_{22}O_4$: C, 72.59; H, 7.05. Found: C, 72.61, 72.57; H, 7.22, 6.90; CH_3CO, 0.

A very small amount of small prisms crystallized together with the large prisms. They were separated mechanically, and yielded after one recrystallization from a mixture of ether and petroleum ether small prisms melting at 199–204° (VII). The product gave a melting point depression with the needles melting at 203–204°.

Anal. Calcd. for $C_{19}H_{18}O_5$: C, 69.92; H, 5.56. Calcd. for $C_{19}H_{20}O_5$: C, 69.50; H, 6.14. Found: C, 70.18, 70.34; H, 6.36, 6.17.

Other similar hydrogenations yielded varying amounts (4–25% by weight) of mixtures of $C_{19}H_{14}O_5$ (VI) and $C_{19}H_{16}O_4$ (VIII), and of the prisms $C_{19}H_{22}O_4$ (X) (25–42%). The yield of X was generally lower when more of the mixture of VI and VIII was obtained. The rest was an oil. The other two crystalline products were not isolated in these experiments. A hydrogenation of the mixture of VI and VIII, under conditions used in the above-described experiments, gave a 60% (by weight) yield of the prisms X.

The oil, that remained after the above-described crystalline products had been isolated, was distilled in high vacuum and analyzed.

Anal. Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.31, 75.25; H, 8.06, 8.22.

Monoacetyl Derivative of $C_{19}H_{16}O_4$ (VIII).—A solution of 0.1 g. of $C_{19}H_{16}O_4$ in 10 cc. of dry pyridine and 5 cc. of acetic anhydride was heated for 2 hours to 90° and was then concentrated *in vacuo* to dryness. The residue was purified by crystallization from a mixture of methylene chloride and petroleum ether. It forms colorless heavy prisms melting at 245–247°.

Anal. Calcd. for $C_{21}H_{18}O_5$: C, 71.99; H, 5.18; acetyl, 12.3. Found: C, 71.85, 71.60; H, 5.21, 5.53; acetyl, 11.19.

Monoacetyl Derivative of $C_{19}H_{14}O_5$ (VI).—The acetylation was carried out in pyridine at 90°, as with $C_{19}H_{14}O_4$, and also at room temperature. The same product resulted. It forms white needles from a mixture of methylene chloride and petroleum ether, melting at 239–241°. The yield was almost quantitative. It gives a yellow color with concentrated sulfuric acid. The product was recovered unchanged after treatment for 20 hours in dimethylformamide with an excess of diazomethane in ether.

Anal. Calcd. for $C_{21}H_{16}O_6$: C, 69.22; H, 4.43; acetyl, 11.8. Found: C, 69.04, 69.14; H, 4.29, 4.20; acetyl, 11.51; methoxyl, 0.

Monomesyl Derivative of $C_{19}H_{14}O_5$ (VI).—To a solution of 0.3 g. of VI in 60 cc. of dry pyridine was added 4 cc. of methanesulfonyl chloride. The mixture was left for 20 hours at room temperature, then treated with ice-water and concentrated *in vacuo*. Water and some dilute hydrochloric acid were added, and the reaction product extracted with methylene chloride. The methylene chloride solution was dried, treated with activated carbon, filtered and concentrated *in vacuo*. The residue was then crystallized from a mixture of methylene chloride and ether. The product forms fine needles or flat prisms melting at 273–274°. Its solution in concentrated sulfuric acid shows a very faint yellow color.

Anal. Calcd. for $C_{20}H_{16}O_7S$: C, 60.00, H, 4.03. Found: C, 60.40, 59.62; H, 3.82, 3.71; methoxyl, 0.

Monomethyl Ether of $C_{19}H_{14}O_5$ (VI).—To a solution of 0.3 g. of VI in 150 cc. of dimethylformamide was added an excess of diazomethane in ether. After 24 hours the mixture was concentrated *in vacuo*. The residue yielded after crystallization from a mixture of chloroform and petroleum ether faintly yellowish needles melting at 240–241°. It gives a melting point depression with the starting material.

Anal. Calcd. for $C_{20}H_{16}O_5$: C, 71.42; H, 4.80; CH_3O, 9.22. Found: C, 71.48, 71.78; H, 4.88, 4.81; CH_3O, 9.69, 9.53.

Monoacetyl Derivative of $C_{19}H_{22}O_4$ (X).—A solution of 0.2 g. of compound X in a mixture of 2 cc. of pyridine and 2 cc. of acetic anhydride was left for 15 hours at room temperature. The mixture was then concentrated *in vacuo*, and the residue crystallized from a mixture of acetone, ether and petroleum ether, yielding colorless prisms melting at 173–177°.

Anal. Calcd. for $C_{21}H_{24}O_5$: C, 70.76; H, 6.79; CH_3CO, 11.7. Found: C, 70.72, 70.97; H, 6.93, 6.76; CH_3CO, 8.65, 8.28.

Alkaline Hydrolysis of $C_{19}H_{14}O_5$ (VI); Formation of $C_{18}H_{16}O_4$ (XII).—To a hot solution of 0.5 g. of VI in 50 cc. of dioxane was added 75 cc. of 1 *N* sodium hydroxide. The mixture was refluxed for 30 minutes, acidified, partly concentrated *in vacuo* and extracted with chloroform. The chloroform solution was dried and concentrated *in vacuo*. The residue was crystallized from methanol, or a mixture of acetone and petroleum ether, and yielded then yellowish needles or plates decomposing at 304–309° (XII). The product dissolves with yellow color in concentrated sulfuric acid. The solution in 3 *N* alkali is yellow, turns dark on contact with air and can be reduced again with zinc dust.

Anal. Calcd. for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 72.96, 73.16; H, 5.32, 5.39.

Diacetyl Derivative of $C_{18}H_{16}O_4$ (XII).—A solution of 0.1 g. of XII in a mixture of 10 cc. of pyridine and 10 cc. of acetic anhydride was left at room temperature for 48 hours. It was then concentrated *in vacuo*, and the residue was crystallized from a mixture of ether and petroleum ether. The product forms fine needles melting at 231–232°.

Anal. Calcd. for $C_{22}H_{20}O_6$: C, 69.46; H, 5.30; acetyl, 22.6. Found: C, 68.90; H, 5.33; acetyl, 18.51, 18.19.

Dimesyl Derivative of $C_{18}H_{16}O_4$ (XII).—To a solution of 0.2 g. of XII in 10 cc. of pyridine was added 1.5 cc. of methanesulfonyl chloride. The mixture was left at room temperature for 2 hours and was then diluted with ice-water. The reaction product was filtered off and recrystallized from a mixture of acetone, ether and petroleum ether. It forms needles melting at 207–208°.

Anal. Calcd. for $C_{20}H_{20}O_8S_2$: C, 53.10; H, 4.46. Found: C, 53.39, 53.07; H, 4.35, 4.39.

Isolation of $C_{18}H_{18}O_3$ (XIII).—To a hot solution of 1 g. of a mixture of hydrogenation products of the aglycone, containing about 40% $C_{19}H_{16}O_4$ (VIII) and 60% $C_{19}H_{14}O_5$ (VI), in 180 cc. of dioxane was added 150 cc. of 1 *N* sodium hydroxide. The mixture was boiled for 1 hour in a stream of nitrogen, distilling off part of the dioxane–water mixture; 100 cc. of 2 *N* hydrochloric acid and ice was then added to the brown solution, and the crystalline gray precipitate was filtered off (XIII, 0.35 g.). After recrystallization from a mixture of ether and petroleum ether, the product formed gray prisms melting at 228–234°. It dissolves with red color in concentrated sulfuric acid, and is soluble with yellow color in warm 3 *N* alkali.

Anal. Calcd. for $C_{18}H_{18}O_3$: C, 76.57; H, 6.43. Found: C, 76.51, 76.96, 76.30; H, 6.38, 6.36, 6.39.

The acidic aqueous solution remaining after the removal of $C_{18}H_{18}O_3$ (XIII) was extracted with ether. The extract yielded after removal of the solvent 0.5 g. of a partly crystalline residue. This product was recrystallized from methanol and proved to be compound XII, $C_{18}H_{16}O_4$, which had been obtained by alkaline hydrolysis of pure compound VI, $C_{19}H_{14}O_5$.

Diacetyl Derivative of $C_{18}H_{18}O_3$ (XIII).—A solution of 0.3 g. of XIII in a mixture of 10 cc. of pyridine and 10 cc. of acetic anhydride was left at room temperature for 48 hours. The mixture was then concentrated *in vacuo*, and the residue crystallized from a mixture of ether and petroleum ether. The product forms colorless prisms melting at 136–137°.

Anal. Calcd. for $C_{22}H_{22}O_5$: C, 72.11; H, 6.05; acetyl, 23.5. Found: C, 71.81, 71.75; H, 5.87, 6.17; acetyl, 20.28, 21.25.

Alkaline Hydrolysis of $C_{19}H_{22}O_4$ (X).—Twenty-five cc. of 3 *N* sodium hydroxide was added to a solution of 0.5 g. of X in 50 cc. of methanol. The mixture was boiled, distilling off the methanol. After 25 minutes the reddish-brown solution (*ca.* 20 cc.) was cooled, diluted with 25 cc. of water,

acidified with concentrated hydrochloric acid, and extracted with ether. The ether extract was dried and concentrated *in vacuo*. The residue was dissolved in ether, purple colored impurities were precipitated by the addition of petroleum ether and filtered off. The ether solution was concentrated to a small volume yielding pink rosettes of prisms (0.45 g.) melting at 148–150°. The crystals were dissolved in methanol, the solution was treated with activated carbon, concentrated *in vacuo*, and the residue crystallized twice from a mixture of ether and petroleum ether. The product thus obtained is an acid. It forms colorless prisms melting at 151–152° and gives a melting point depression with compound X, $C_{19}H_{22}O_4$.

Anal. Calcd. for $C_{19}H_{24}O_5$: C, 68.65; H, 7.28; equiv. wt., 332.28. Found: C, 68.53, 68.78; H, 7.29, 7.56; equiv. wt., 333.2, 328.6.

Refluxing with chlorobenzene resulted in elimination of 1 mole of water and reconversion into $C_{19}H_{22}O_4$ (X). Treatment with acetic anhydride in pyridine at room temperature resulted in the formation of the previously described monoacetyl derivative of X.

Dehydrogenation of Reduced Aglycone Derivatives.—Twelve grams of the oily mixture XI, which analyzed approximately for $C_{19}H_{24-26}O_3$ and was obtained by hydrogenation of the aglycone with PtO_2, was heated to 320–350° for 10.5 hours with 10 g. of 10% palladium–carbon catalyst. The cooled mixture was extracted with a large amount of ether. The ether extract was partly concentrated, yielding 0.3 g. of yellow crystals (plates) melting at 194–196° (A). The mother liquor was concentrated to dryness, and gave an oily residue which, after dissolving in hot alcohol, yielded 0.26 g. of yellow crystals melting at 162–167° (B). The alcoholic solution was concentrated *in vacuo*, and the remaining oil (2.6 g.) was distilled in a three-bulb tube at a vacuum of 0.5 mm. and an air-bath temperature of 140–165°. This gave 1.8 g. of a brownish viscous oil (C).

2,4,7-Trinitrofluorenone Derivative of x-Methyl-2,3-benzofluorene.—Fraction B (0.26 g., m.p. 162–167°), obtained in the above-described dehydrogenation experiment, was purified by crystallization from alcohol, yielding 0.13 g. of crystals melting at 182–183°. This product was combined with fraction A (0.3 g., m.p. 194–196°), the mixture was dissolved in hot acetone and added to a hot acetone solution of 0.6 g. of 2,4,7-trinitrofluorenone (TNF). The mixture was cooled to +5° for 15 hours and then filtered, yielding 0.87 g. (corresponding to 0.37 g. of hydrocarbon) of orange needles melting at 222–224°. After crystallization from acetone, the product melted at 225–226°. The TNF derivative prepared from commercial 2,3-benzofluorene (Aldrich Chemical Co.) melted at 219–220°,[33] and a mixture of the two melted at 217–218°.

Anal. Calcd. for $C_{31}H_{19}O_7N_3$: C, 68.25; H, 3.51; N, 7.70. Found: C, 68.53, 68.42; H, 3.71, 3.68; N, 7.90, 7.94.

x-Methyl-2,3-benzofluorene, $C_{18}H_{14}$.—The TNF derivative (0.85 g., m.p. 222–224°, equiv. to about 0.36 g. of hydrocarbon) was dissolved in warm benzene and decomposed on a column containing 130 g. of activated alumina (Harshaw). The hydrocarbon was eluted with benzene. The first fractions of hydrocarbon were completely white (0.17 g.), then followed a yellowish fraction (0.1 g.). The rest was contaminated with 2,4,7-trinitrofluorenone and was rechromatographed, giving an almost quantitative recovery of the hydocarbon. The yellowish fraction (0.1 g.) was dissolved in petroleum ether and freed from the yellow contaminant by chromatographic purification using 40 g. of alumina as adsorbent and petroleum ether as eluent. The white hydrocarbon was recovered in practically quantitative yield.

The white hydrocarbon fractions were combined and purified by dissolving in ether, followed by addition of ethanol and evaporation of the ether by heating at atmospheric pressure. Repeated recrystallization in this manner resulted in colorless fine plates melting at 222–222.5°. A mixture with purified commercial, 2,3-benzofluorene,[34] melting at 212–213,[33] showed a melting point of 210–211°. A very pure sample of our hydrocarbon was converted into the TNF derivative, and gave again a product melting at 225–226°. The hydrocarbon *does not* form a picrate in chloroform like 2,3-benzofluorene. The ultraviolet absorption spectrum (Fig. 2) shows the close relationship to 2,3-benzofluorene; the infrared spectrum shows differences in the strong absorption bands in the 690–900 cm.$^{-1}$ region.

Anal. Calcd. for $C_{18}H_{14}$: C, 93.87; H, 6.13. Found: C, 93.97; H, 6.05.

2,4,7-Trinitrofluorenone Derivative of x-Methyl-2-phenylnaphthalene.—The distillate C (1.8 g.) of the above-described dehydrogenation experiment was dissolved in a small amount of hot acetone and was combined with a hot saturated acetone solution of 1.8 g. of TNF. The mixture yielded after cooling 0.51 g. of red needles melting at 194–200°. This material consisted mostly of the TNF derivative of x-methyl-2,3-benzofluorene described above and corresponds to about 0.2 g. of hydrocarbon. The mother liquors of this TNF derivative deposited on further concentration, in addition to 0.78 g. of unreacted TNF, different fractions of a lower melting TNF derivative. Together, 5 fractions (0.8 g., corresponding to 0.32 g. of hydrocarbon) were obtained, which melted between 134 and 140°. In addition, 0.2 g. of a very impure TNF derivative also was obtained. The product melting between 134–140° was repeatedly recrystallized from alcohol and yielded finally fine orange needles melting at 142–143°.

Anal. Calcd. for $C_{30}H_{19}O_7N_3$: C, 67.54; H, 3.59; N, 7.88. Found: C, 67.14, 67.35, 67.51, 67.65; H, 3.82, 3.72, 3.47, 3.33; N, 8.07, 8.28.

x-Methyl-2-phenylnaphthalene, $C_{17}H_{14}$.—A solution of 217 mg. of the above TNF derivative (m.p. 138–140°) in a mixture of equal parts of benzene and petroleum ether was decomposed on a column of 20 g. of activated alumina. The hydrocarbon was eluted with a mixture of benzene and petroleum ether (50:50); 83 mg. (calcd. 88.7 mg.) of a liquid colorless hydrocarbon was obtained. The product solidifies at 0° and liquefies again at room temperature. Its ultraviolet absorption spectrum (Fig. 3) indicates its close relationship to 2-phenylnaphthalene.

Anal. Calcd. for $C_{17}H_{14}$: C, 93.53; H, 6.47. Found: C, 92.98, 92.89; H, 6.91, 6.95.

x-Methyl-2-phenylnaphthalene Complex with Two Moles of 1,3,5-Trinitrobenzene.—To 76 mg. of the above $C_{17}H_{14}$ hydrocarbon in a small amount of alcohol was added a hot alcoholic solution of 76 mg. (1 mole) of trinitrobenzene (TNB). The mixture was partly concentrated *in vacuo*, the precipitated TNB derivative was filtered off and recrystallized from hot alcohol. The product forms yellow plates melting at 102–103°.

Anal. Calcd. for $C_{29}H_{20}O_{12}N_6$: C, 54.04; H, 3.13; N, 13.04. Found: C, 54.22, 54.07; H, 2.97, 3.30; N, 13.32.

In the above-described dehydrogenation experiment, a total of 0.63 g. (2.75 moles) of x-methyl-2,3-benzofluorene was obtained from 12 g. (about 40 moles) of starting material, which corresponds to a yield of 7%; in addition 0.32 g. of x-methyl-2-phenylnaphthalene was obtained, corresponding to 3.7%. A relatively large fraction of the dehydrogenation product (0.7 g.) did not react with TNF and was recovered by chromatographic purification. It did not form a trinitrobenzene derivative either.

A six-hour dehydrogenation experiment of the same starting material yielded 3.2% of the 2,3-benzofluorene and 1.8% of the 2-phenylnaphthalene derivative. A dehydrogenation of the crystalline product $C_{19}H_{22}O_4$ yielded 2% of the x-methyl-2,3-benzofluorene and 3.9% of the x-methyl-2-phenylnaphthalene.

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(33) M. Orchin, L. Reggel and E. Woolfolk, THIS JOURNAL, **69**, 1225 (1947), report a melting point of 212.2–213.8° for 2,3-benzofluorene and 221.2–222° for its TNF derivative.

(34) The commercial product was purified by conversion into the picrate or TNF derivatives, crystallization of the derivative, reconversion into the hydrocarbon and crystallization of the hydrocarbon.