[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

The Choleic Acids of Certain Carcinogenic Hydrocarbons

BY LOUIS F. FIESER AND MELVIN S. NEWMAN

The remarkable ability of desoxycholic acid to form well-defined molecular compounds (choleic acids) with a wide variety of organic substances was discovered by Wieland and Sorge,[1] who showed that this bile acid, either in the free state or in the form of glyco- or tauro-desoxycholic acid, combines with such substances as ether, alcohol, various carboxylic acids including the higher fatty acids, certain aromatic hydrocarbons, cholesterol, certain alkaloids and other compounds. The observation that water-insoluble substances can be brought into aqueous solution in the form of sodium salts of the choleic acids suggested an explanation of the dissolving power of the bile and hence of an important physiological function of the bile acids.

With the idea that water-soluble salts of choleic acids from carcinogenic hydrocarbons might open up various interesting lines of biological experimentation, we undertook the preparation of such compounds. Apparently with the same aim in view, Winterstein and Vetter[2] recently treated 1,2-benzpyrene in acetone with a 20% aqueous solution of sodium desoxycholate and found that, after removal of the acetone, the hydrocarbon had dissolved to the extent of 2 mg. per cc. It seemed to us of more interest to attempt to prepare and characterize the pure choleic acids. Wieland and Sorge[1] found that xylene combines with two molecules of desoxycholic acid and that naphthalene forms a 1:2 compound (1 mole of hydrocarbon to 2 moles of desoxycholic acid), but since data concerning other polynuclear hydrocarbons are lacking, a survey was made of the ability of some of the prominent members of the series to combine with desoxycholic acid.

The method consisted in dissolving the hydrocarbon with 10–15 parts by weight of desoxycholic acid (m. p. 172–173°, corr.)[3] in hot absolute alcohol and observing the character of the crops of crystals which separated on slow cooling of the solution at various stages of concentration. The formation of a choleic acid usually was clearly evident from the appearance of the crystals as compared with those of the hydrocarbon, and a definite indication was afforded by the sharp melting points of these extraordinary molecular compounds. With the following compounds the results were negative, the hydrocarbon in each case crystallizing unchanged from the alcoholic solution: anthracene, naphthacene, chrysene, pyrene, fluoranthene, triphenylene, perylene, 1,2-benzpyrene (m. p. 178.8–179.3°, corr.),[4] 4′-methyl-1,2-benzpyrene (m. p. 219.5–220°, corr.),[4] and 4′-methyl-1′,2′-dihydro-1,2-benzpyrene (m. p. 162–163°, corr.).[4] In the case of anthracene, naphthacene and fluoranthene there was some slight indication of compound formation, but sufficient material for characterization was not obtained.

The hydrocarbons from which pure choleic acids were obtained are indicated in the table. Usually several crops of the addition compound were collected and the total yield was about 50%. The acenaphthene and phenanthrene choleic acids could be recrystallized from alcohol, but the other compounds could not be recrystallized from this solvent without considerable loss and change in composition, even in the presence of some excess desoxycholic acid. Alcohol is not a good solvent for the polynuclear hydrocarbons, but it has the advantage over other common solvents of having less affinity for desoxycholic acid. Dioxane was the only other solvent which was found to be of service and it was useful in only one case. 1,2,5,6-Dibenzanthracene formed a choleic acid better in dioxane than in alcohol, an almost quantitative yield being obtained easily with the former solvent. The choleic acid also can be recrystallized from dioxane. In every other case the only compound which crystallized from this solvent was dioxane-choleic acid which, when freshly prepared, melts at 173.5–174.5°, corr.

The coördination number, or the number of molecules of desoxycholic acid found in combination with the hydrocarbon, was determined by the titration of a sufficiently large sample of the molecular compound with methyl alcoholic potassium hydroxide. This method seemed more

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[1] Wieland and Sorge, *Z. physiol. Chem.*, **97**, 1 (1916).

[2] Winterstein and Vetter, *ibid.*, **230**, 169 (1934).

[3] Purified by Sobotka's method, *Biochem. J.*, **26**, 555 (1932).

[4] These constants were found for samples prepared as previously described [THIS JOURNAL, **57**, 782 (1935)], but further purified by the chromatographic adsorption method and by crystallization.

TABLE I

CHOLEIC ACIDS

From	M. p., °C. corr.[a]	Formula	Titrations[b]				Molecular weight		
			Substance, g.		0.1 N HCl equiv., cc.		Found		Calcd.
Acenaphthene	175.5–176.5	$C_{12}H_{10}\cdot(C_{24}H_{40}O_4)_2$	1.1154	1.0643	23.68	22.38	942	951	939
Phenanthrene	184–185	$C_{14}H_{10}\cdot(C_{24}H_{40}O_4)_3$	0.4128	0.4737	9.10	10.36	1361	1372	1356
1,2-Benzanthracene	198–199	$C_{18}H_{12}\cdot(C_{24}H_{40}O_4)_3$	.1243	.1641	2.69	3.52	1386	1367	1406
1,2,5,6-Dibenzanthracene A[c]	223–224	$C_{22}H_{14}\cdot(C_{24}H_{40}O_4)_4$	.1021	.0938	2.20	2.04	1856	1839	1848[e]
B[d]			.5748	.5026	12.54	10.79	1834	1863	
Methylcholanthrene[f]	193.5–194.5	$C_{21}H_{16}\cdot(C_{24}H_{40}O_4)_4$	.2271	.3884	4.88	8.34	1861	1863	1838
Hexahydromethylcholanthrene[g]	191.5–192.5	$C_{21}H_{22}\cdot(C_{24}H_{40}O_4)_4$	.5957	.5751	12.75	12.45	1869	1848	1844

[a] With sintering at temperatures 1–5° lower. [b] Using an excess of approximately 0.1 N methyl alcoholic potassium hydroxide, diluting, and back titrating with 0.1072 N HCl. [c] From alcohol, m. p. 223–224°, corr. Microcombustion (Mrs. G. M. Wellwood). Calcd.: C, 76.66; H, 9.49. Found: C, 76.84; H, 9.92. (Calcd. for the 1:3 compound: C, 77.53; H, 9.28. Calcd. for the 1:5 compound: C, 76.09; H, 9.63.) [d] From dioxane, m. p. 221.5–222.5°, corr. (no depression with Sample A). [e] Calculated for the 1:3 compound, 1456; for the 1:5 compound, 2240. [f] Microcombustion (G. M. W.). Calcd.: C, 76.42; H, 9.65. Found: C, 76.61; H, 9.72. [g] Prepared according to Wieland and Dane [*Z. physiol. Chem.*, **219**, 240 (1933)], m. p. 156–157°, corr.

accurate and more reliable than combustion analysis, as the combustions presented certain uncontrolled difficulties. As in the case of other series of choleic acids which have been studied,[5] the numbers conform to the coördination principle, and the number of molecules of desoxycholic acid held in coördinate combination increases with the number of carbon atoms in the acholic constituent. The degree of unsaturation does not appear to be a factor of great importance, for methylcholanthrene and its hexahydro derivative have the same coördination number. In this connection it may be noted that hexadecane and dodecane form choleic acids (m. p. 192–193°, 186–187°, corr., respectively). As might be inferred from previous work,[6] hexamethylethane does not combine with desoxycholic acid in alcoholic solution.

It is doubtful if much significance can be attached to the formation or non-formation of a choleic acid under the conditions of our experiments. Whether the molecular compound or the hydrocarbon separates from a solution of the components depends upon the relative solubilities of the two components and of the choleic acid in the mixture and upon the degree of association of the coördinate compound. With any solvent but alcohol, the solubility and degree of association of the solvent-choleic acid probably also determine the outcome. The non-formation of choleic acids from several of the polynuclear hydrocarbons probably is the result of an unfavorable combination of factors. Combination might take place if a suitable solvent could be found. Although 1,2-benzpyrene fails to unite with desoxycholic acid in alcoholic solution, the experiment of Winterstein and Vetter[1] shows that acholeic acid can be obtained by another method of synthesis.

Dr. Egon Lorenz will report later the results of a study of the absorption spectra of methylcholanthrene-choleic acid and 1,2,5,6-dibenzanthracene-choleic acid. In each case the dilute solution in alcohol or ether gives the spectrum of the pure hydrocarbon, indicating that the molecular compound is completely dissociated. With a solution of either addition compound in dilute, aqueous alkali the absorption spectrum from 3000 Å. to longer wave lengths has a pattern closely related to that of the hydrocarbon in organic solvents (sharp bands), but shifted in the direction of longer wave lengths. In the region below 3000 Å. the spectrum is quite different, for bands which seem to correspond to those for the hydrocarbon are much less distinct and lie in the region of shorter wave length.

The choleic acids of the two carcinogenic hydrocarbons, as well as their water-soluble sodium salts, are being tested for carcinogenic activity by Dr. M. J. Shear. Thus far the subcutaneous injection of the crystalline acids has not proved lethal to mice, although administration of uncombined desoxycholic acid in large doses proved fatal. With methylcholanthrene–choleic acid ulceration and tissue proliferation have occurred in the same way and after the same interval as with methylcholanthrene. The first definite tumor was noted on the sixty-third day.

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(5) Rheinboldt, *Ann.*, **451**, 256 (1926); Sobotka and Goldberg, *Biochem. J.*, **26**, 557 (1932).

(6) Sobotka and Goldberg, *ibid.*, **26**, 566 (1932); Chargaff and Abel, *ibid.*, **28**, 1901 (1934).

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Summary

Molecular compounds containing from two to four molecules of desoxycholic acid have been prepared from certain polynuclear aromatic hydrocarbons, including the carcinogenically active methylcholanthrene and 1,2,5,6-dibenzanthracene. Since the sodium salts of these choleic acids are soluble in water, this provides a means of obtaining aqueous solutions containing carcinogenic hydrocarbons.

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The Reactions of Titanium and Manganese with Hydrogen Peroxide in Basic Solution[1]

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In the usual scheme of qualitative analysis for the common and the rarer metals, titanium appears in the iron division of the ammonium sulfide group.[2] The manganese is removed as the dioxide by boiling with nitric acid and potassium chlorate. Unless the ratio of titanium to manganese is very small, most of the former is left in solution with the iron, etc. The iron may then be precipitated by the use of sodium phosphate, hydrogen peroxide and sodium hydroxide, leaving the titanium in a soluble peroxidized form.[3] A similar action takes place when the phosphate is omitted. If, however, the manganese has not been previously removed, some or all of the titanium is precipitated during the process. This paper describes a study of this reaction.

Solutions of known and varying concentrations of manganese and titanium nitrates were made up. These were treated with excess hydrogen peroxide and with one normal sodium hydroxide until neutral and with 5 ml. in excess. A mahogany brown color appeared which was followed by the formation of a fine grayish-brown precipitate which was shown to contain all of the manganese. In the quantitative tests the titanium was determined in the solution after filtration, and its amount in the residue calculated by difference. Qualitative tests showed that both manganese and titanium appeared in the precipitate but that titanium was present in the filtrate only when the molar ratio of titanium to manganese exceeded approximately two. Excess peroxide was found present in all cases. Whenever the residue was dissolved in acid, the yellow or orange color of peroxidized titanium appeared, which is not the case when a mixture of $Ti(OH)_4$ and freshly precipitated manganese dioxide is similarly treated. This would indicate the presence of peroxidized titanium in the residue.

A series of quantitative tests was made in which the molar ratio of titanium to manganese in the original solutions varied between 2.2 and 8.4. The corresponding ratios in the resulting precipitates were found to vary between 1.8 and 4.8, indicating that no definite compound is formed, but that a greater amount of titanium is precipitated the larger its initial proportion. When the titanium and manganese solutions were not mixed until each had been treated separately with peroxide and sodium hydroxide, a similar result was obtained, the ratio of titanium to manganese in the residue being about half that obtained for corresponding concentrations in the previous series.

When the two metals were precipitated together by peroxide and sodium hydroxide, the ratio of titanium to manganese increased with lapse of time. In a series in which the original molecular ratio of titanium to manganese was 3.6, that in the residue increased steadily from 1.6 to 2.7 during the first twelve hours at 25°. At higher temperatures more titanium was precipitated. In both of these cases, it is probable that some $Ti(OH)_4$ was precipitated due to a partial decomposition of the peroxide, although an excess of the latter was always found in the filtrate. Furthermore, when the other factors were kept constant, the ratio of titanium to manganese in the residue decreased in general with increased concentration of hydrogen peroxide.

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(1) From a master's thesis submitted by G. N. Cade, Jr., in partial fulfilment of the requirements for a Master's degree at the University of Arkansas, 1935. Research Paper No. 390, Journal Series, University of Arkansas.

(2) A. A. Noyes, W. C. Bray and E. B. Spear, THIS JOURNAL, **30**, 481 (1908).

(3) P. E. Browning, G. S. Simpson and L. E. Porter, *Am. J. Sci.*, **42**, 106 (1916).