A COMPANION OF CHOLESTEROL

Sir:

Evidence of low-order carcinogenicity of a variety of cholesterol-rich lipid fractions[1] and of high carcinogenic potency[2] of Spielman and Meyer's[3] crude progesterone preparation derived from cholesterol by bromination, oxidation, and debromination suggests the existence of a possibly endogenous non-aromatic steroid carcinogen related to or derived from cholesterol. The hypothesis that the substance is an abnormal ester of cholesterol will be discussed in a paper with Dr. W. P. Schneider. Consideration of the alternate possibility that the carcinogen is a product of oxidation of cholesterol, perhaps a variant of one of the hormone structures, prompted a restudy of oxidation with hexavalent chromium and with selenium dioxide. In the former instance experimentation was greatly facilitated by use of a solution of sodium dichromate dihydrate in glacial acetic acid; with addition of 1 volume of benzene per 2 volumes of acetic acid, oxidations can be conducted in homogeneous solution at 0°. The four known oxidation products were all isolated;[4] Δ^5-cholestenone was found to be the precursor of the chief product, Δ^4-cholestene-3,6-dione; a new product is a cholestenedione, m.p. 189°, dec., $[\alpha]^{23}D$ +27° Chf, +31° Di; $\lambda_{max.}^{Chf}$ 5.85, 6.23 μ; $\lambda_{max.}^{EtOH}$ 236 mμ (log ϵ 4.16); found: C, 81.46; H, 10.82.

The observation that in both the dichromate and selenium dioxide oxidations of commercial cholesterol (Wilson), chromatography led to isolation of small amounts of substances that did not appear to be derivable from cholesterol prompted investigation of the homogeneity of the starting material. Repeated crystallization of the acetic acid complex effected slow distribution of companion steroids into the mother-liquor fractions that eventually sufficed for isolation of cholestanol as acetate, m.p. and mixed m.p. 110–111°, found: C, 80.75; H, 11.65, following bromination, and, by chromatography of the acetate mixture, of an isomer of cholesterol, m.p. 125–126°, $[\alpha]^{27}D$ + 5.7° Chf, found: C, 83.70; H, 12.15; acetate, m.p. 118–119°, $[\alpha]^{22}D$ + 1.5° Chf, found: C, 81.07; H, 11.50. This substance and its acetate showed no depression when mixed with Δ^7-cholesterol, m.p. 125–126°, $[\alpha]^{23}D$ + 3.9° Chf, +10.0° Di, and its acetate, m.p. 118–119°, $[\alpha]^{23}D$ + 2.4° Chf, +9.4° Di; it is thus 5,6-dihydroprovitamin D_3.[5]

Δ^7-Cholestenol[5] is characterized by high sensitivity to oxidation. A solution at 25° of 1 mg. of material in 0.5 cc. of benzene plus 0.5 cc. of 0.1 *M* selenious acid in aqueous acetic acid turns yellow in 2–3 minutes and deposits red selenium in 10–15 minutes; the reaction is rapid even at 0°. The test appears to be positive only for steroids of the A/B-*trans* series having a double bond or a dienic system adjacent to a 14α-hydrogen atom. Kogi Nakanishi has developed a microanalytical procedure based on this reaction applicable to a 5–15 mg. sample; some results for % Δ^7-stenol in cholesterol samples processed by Dr. Bidyut Bhattacharyya are: spinal-cord (Wilson): 0.62; beef adrenal, 0.65; liver, 0.35; normal plasma, 0.42–1.35; gall stone, 2.19–3.11; wool fat, 2.97; egg yolk, 4.34; cholesterol purified through the dibromide or by 22 crystallizations from acetic acid, 0.0.

Treatment of Δ^7-cholestenyl acetate with 2 moles of NBS in ether–methanol gave, in 27% yield, $\Delta^{7,9(11)}$-cholestadienyl acetate, m.p. and mixed m.p. 118–119°, $\lambda_{max.}^{EtOH}$ 236, 243, 250 mμ (log ϵ 4.10, 4.15, 3.96), found: C, 81.47; H, 10.69.[6] Since NBS in methanol is equivalent to bromine, it can be inferred that in the Spielman–Meyer process the Δ^7-cholestenol present was converted initially into $\Delta^{7,9(11)}$-cholestadienol and hence that the carcinogen may be an oxidation pronuct of this diene alcohol. A number of products of oxidation of $\Delta^{7,9(11)}$-dienes have been reported,[7] among them a monoepoxide.[7b] In view of the demonstrated carcinogenicity of the diepoxide of vinylcyclohexene,[8] it seems possible that the substance may be 7,8,9,11-diepoxycholestanol. This conceivably could be formed in lard-injected cholesterol[1] by the action of peroxides of lard on lathosterol; cholesterol administered in sesame oil, which contains a natural antioxidant preventing peroxidation, has given no tumors.[2]

(1) I. Hieger, *Brit. J. Cancer*, **3**, 123 (1949).

(2) F. Bischoff and J. J. Rupp, *Cancer Research*, **6**, 403 (1946).

(3) M. A. Spielman and R. K. Meyer, THIS JOURNAL, **61**, 893 (1939).

(4) The formation of "α-oxycholestenol," J. Mauthner and W. Suida, *Monatsh.*, **17**, 579 (1896), has not previously been confirmed. My material had the constants: m.p. 187.5–188°, $[\alpha]D$ −6° Di, −10° Chf, $\lambda_{max.}^{EtOH}$ 237 mμ(log ϵ 3.83).

(5) The term lathosterol (Gr. *latho-*, undetected) for this substance is in use in our laboratory to describe material of biological origin.

(6) J. E. Herz has established that Δ^7-cholestenyl benzoate can be substituted for the $\Delta^{7,9(11)}$-diene in our previously described process for C_{11}-oxygenation with NBS, Ref. 7d.

(7) (a) L. F. Fieser and co-workers, THIS JOURNAL, **73**, 2397 (1951); (b) E. M. Chamberlain, *et al.*, *ibid.*, **73**, 2396 (1951); (c) G. Stork, J. Romo, G. Rosenkranz and C. Djerassi, *ibid.*, **73**, 3546 (1951); (d) L. F. Fieser and co-workers, *ibid.*, **73**, 4053 (1951).

(8) J. A. Hendry, R. F. Homer, F. L. Rose and A. L. Walpole, *Brit. J. Pharmacol. and Chemotherap.*, **6**, 235–255 (1951).

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RECEIVED SEPTEMBER 6, 1951

THE SYNTHESIS OF 5-HYDROXYTRYPTAMINE

Sir:

The isolation[1] of the substance believed to be responsible for the vasoconstrictor activity of serum and the proposal[2] that the active principle is 5-hydroxytryptamine prompted this investigation.

5-Benzyloxyindole[3] on treatment with formaldehyde and dimethylamine gave 5-benzyloxygramine, m.p. 138° (*Anal.* Calcd. for $C_{18}H_{20}N_2$: C, 77.09; H, 7.19; N, 9.99. Found: C, 77.38; H, 7.02; N, 10.01). Using the procedure of Snyder,[4] this Mannich base was heated with sodium cyanide in aqueous ethanol to yield 5-benzyloxyindole-3-acetamide, m.p. 158° (*Anal.* Calcd. for $C_{17}H_{16}N_2O_2$: C, 72.83; H, 5.75; N, 10.00. Found: C, 72.93; H, 5.78; N, 10.27). The acetamide on reduction

(1) M. M. Rapport, A. A. Green and I. H. Page, *J. Biol. Chem.*, **176**, 1243 (1948).

(2) M. M. Rapport, *ibid.*, **180**, 961 (1949).

(3) H. Burton and J. L. Stoves, *J. Chem. Soc.*, 1726 (1937).

(4) H. R. Snyder and F. J. Pilgrim, THIS JOURNAL, **70**, 3770 (1948).

with lithium aluminum hydride produced the corresponding amine, 5-benzyloxytryptamine, which was isolated as the hydrochloride, m.p. 265° (*Anal.* Calcd. for $C_{17}H_{18}N_2O\cdot HCl$: C, 67.43; H, 6.33; N, 9.26. Found: C, 67.39; H, 6.22; N, 9.32). Catalytic debenzylation of this amine hydrochloride afforded the desired 5-hydroxytryptamine hydrochloride, a light-sensitive hygroscopic salt, m.p. 167–168° (*Anal.* Calcd. for $C_{10}H_{20}N_2O\cdot HCl$: C, 56.47; H, 6.16; N, 13.18. Found: C, 56.07; H, 6.20; N, 12.94). The picrate, formed in water from the hydrochloride, melted (Fischer–Johns apparatus) from 103–111°, resolidified at 124–134° and remelted from 185–189° (*Anal.* Calcd. for $C_{10}H_{12}N_2O\cdot C_6H_3N_3O_7\cdot H_2O$: C, 45.40; H, 4.05; N, 16.55. Found: C, 45.20; H, 3.94; N, 16.62). The absorption spectrum of 5-hydroxytryptamine in aqueous solution at *p*H 5.4 has a maximum at 2750 Å., a shoulder with a point of inflection at 2990 Å., and a minimum at 2500 Å. At *p*H 11.6, the position of the maximum at 2750 Å. is essentially unchanged while the second peak shifts from 2990 to 3220 Å. The data on the picrate and the absorption data on the hydrochloride are in excellent agreement with that published by Rapport[2] for the vasoconstrictor principle, serotonin.

Preliminary pharmacological investigation has shown 5-hydroxytryptamine to have vasoconstrictive properties.

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RECEIVED AUGUST 16, 1951

NINE OR MORE LIQUID PHASES

Sir:

The discovery of an ever-increasing number of incompletely miscible liquid phases has furnished an interesting challenge to physical chemists, not without value in that it has served to direct attention to the variety of factors which can contribute to immiscibility. In 1934 I exhibited a system of five stable liquid phases; in 1940 a sixth was added, and in 1949 a seventh.[1] In 1950 Kittsley and Goeden[2] added to the former set an eighth, a silicone oil; this owes its low solubility in the other liquids to its large cross-linked molecules. I wish now to point out that the "incompatibility" of different high polymers may be invoked to split a liquid in which they are soluble into two or even more liquid layers. Dobry and Boyer-Kawenoki[3] have made an experimental study of a number of such systems and Stockmayer[4] and Scott[5] have given their theoretical interpretation.

For example, the water layer of the previous set can be split into two by using two incompatibles reported by Dobry and Boyer-Kawenoki, methyl cellulose and polyvinyl alcohol, yielding nine layers and there seems no reason to doubt that the same principle could be applied to yield almost unlimited further splitting of any layer for which sufficiently soluble high polymers of different molecular weights and configurations can be found.

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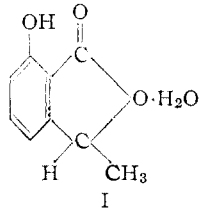
RECEIVED AUGUST 30, 1951

(1) J. H. Hildebrand, *J. Phys. Colloid Chem.*, **53**, 944 (1949).
(2) S. L. Kittsley and H. A. Goeden, THIS JOURNAL, **72**, 4841 (1950).
(3) A. Dobry and F. Boyer-Kawenoki, *J. Polymer Sci.*, **2**, 90 (1947).
(4) W. H. Stockmayer, ACS Meeting, Atlantic City, N. J., April, 1949.
(5) R. L. Scott, *J. Chem. Phys.*, **17**, 279 (1949); see also J. H. Hildebrand and R. L. Scott, "Solubility of Nonelectrolytes," 3rd Edition, Reinhold Publishing Corp., New York, N. Y., 1950; H. Tompa, *Trans. Faraday Soc.*, **45**, 1142 (1949).

A DEGRADATION PRODUCT OF TERRAMYCIN

Sir:

The hydrolysis of the antibiotic terramycin, $C_{22}H_{24-26}N_2O_9$, in hot 20% sodium hydroxide in the presence of zinc has been previously reported[1] to yield terracinoic acid ($C_{13}H_{12}O_6$), ammonia, dimethylamine, acetic acid, carbon dioxide, and a phenolic lactone, $C_9H_8O_3\cdot H_2O$, m.p. 110–112°. *Anal.* Calcd. for $C_9H_8O_3\cdot H_2O$: C, 59.33; H, 5.54; H_2O, 9.89. Found: C, 59.32; H, 5.79; H_2O (K.F.) 9.30. The structure of the phenolic lactone has been shown by degradation and by synthesis to be 7-hydroxy-3-methylphthalide (I).



I

This phthalide is insoluble in bicarbonate and slowly soluble in cold aqueous sodium hydroxide. It gives a purple color with ferric chloride and a positive aminoantipyrine test. Titration of anhydrous I at room temperature shows it to be a monobasic acid with *pK* 8.5 and equivalent weight 162 (calcd. 164). A drop in *p*H occurs when the titrated solution is heated to 100° for one hour indicating the presence of a lactone. In hot sodium ethoxide, I yields an alcohol insoluble crystalline disodium salt of the free acid. *Anal.* Calcd. for $C_9H_8O_4Na_2\cdot H_2O$: C, 44.30; H, 4.12; Na, 18.79. Found: C, 44.00; H, 4.34; Na, 18.45.

Methylation with diazomethane yields a monomethyl ether, m.p. 73–74°, which is very slowly soluble in cold alkali. *Anal.* Calcd. for $C_{10}H_{10}O_3$: C, 67.46; H, 5.76; methoxyl, 17.41. Found: C, 67.45; H, 5.65; methoxyl, 18.0. This methyl ether forms a crystalline alcohol soluble monosodium salt.

Oxidation of the methyl ether of I by potassium permanganate in strongly alkaline solution yields a small amount of 3-methoxyphthalic acid, which has been identified as its anhydride. Fusion of 7-hydroxy-3-methylphthalide (I) with alkali yields salicylic acid, m.p. 159–60°, and acetic acid, identified through its *p*-nitrobenzyl ester, m.p. 77–78°. Cleavage of the aromatic ring-to-carbon bond to yield benzoic acids and aliphatic acids is characteristic of 3-monoalkylated phthalides. This cleavage suggests that the phenolic hydroxyl of I is in the 7 position. This assignment was also favored

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