0.4M concentration. In these mixtures, ceric ion is reduced by H, HO_2, HCOO and H_2O_2 while cerous ion is oxidized by OH and HSO_4.

The experimental data are fairly well represented by the equation

$$G(Ce^{+++}) = 2G_{H_2O_2} + G_H - G_{OH} + 2G_{OH}\Big/\left[1 + \frac{k_1(Ce^{+++}) + k_3(H_2SO_4)}{k_2(HCOOH)}\right]$$

It is assumed in this equation that (a) HSO_4 radical oxidizes cerous ion but does not react with formic acid and (b) HCOO radical reduces[4,5] ceric ion but does not oxidize cerous ion. The previously reported[6] values of G_H = 3.70, G_{OH} = 2.92, G_{H_2} = 0.39 and $G_{H_2O_2}$ = 0.78 are used with a correction made for the decrease in $G_{H_2O_2}$ (with a concomitant increase in G_{OH}) by cerious ion[7] and formic acid. The equation fairly well represents the data with values for k_1/k_2 of 1.70, k_2/k_3 of 380 and k_1/k_3 of 650.

The decreased reactivity of hydrogen with OH radical in 0.4M sulfuric acid must then be attributed to competition of sulfuric acid with hydrogen for reaction with OH radical. The occurrence of reaction 3 in sulfuric acid solutions must of necessity be considered in those cases where the reactions of OH radical differ from those of the radical formed in reaction 3.

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RECEIVED MARCH 5, 1956

2α-HYDROXYLATION OF CORTISOL IN THE GUINEA PIG[1]

Sir:

Recently the isolation of 6β-hydroxycortisol and the partial characterization of two other $C_{21}O_6$ urinary metabolites of cortisol (I) (hydrocortisone) in the guinea pig has been described.[2] 6β-Hydroxycortisol and one of the partially characterized steroids, termed[2] steroid IIa, have also been isolated from the urine of untreated guinea pigs[3] and in markedly elevated concentrations from the urine of guinea pigs with leukemia and liposarcoma.[4] The purpose of this communication is to report on the identification of steroid IIa as 2α-hydroxycortisol (II). The identification was achieved by elemental analysis of the diacetate, by spectroscopic evidence in alkali and by comparison with synthetic II obtained as the major $C_{21}O_6$ product from the reaction of I-21-acetate with lead tetraacetate. This finding represents the first instance of 2α-hydroxylation in a mammal. The only steroids with a hydroxyl at C-2 hitherto found in nature have been known to occur among the sapogenins from plant origin.

Steroid IIa diacetate was isolated, as previously described,[2] from a pool of guinea pig urine. After five crystallizations from methanol an analytical sample was obtained, m.p. 224–230°, $\lambda_{max.}^{methanol}$ 242 mμ (16,000). *Anal.* Calcd. for $C_{25}H_{34}O_8$: C, 64.92; H, 7.41; Found: C, 64.91; H, 7.60. The infrared spectrum and the spectrum in sulfuric acid have been reported previously.[2] The diacetate was hydrolyzed with $KHCO_3$ under the conditions described by Sondheimer, *et al.*[5] Chromatography of the reaction mixture on paper in the chloroform–formamide system gave the free steroid IIa which was identical (running rate on paper, infrared spectrum and spectrum in sulfuric acid) with steroid IIa isolated directly from guinea pig urine. The latter after extensive chromatographic separation and crystallization from ethyl acetate–benzene, exhibited m.p. 185–190°, ν^{KBr} −3300 (hydroxyl), 1704 (C-20 carbonyl), 1669 (C-3 carbonyl), 1616 (Δ⁴-double bond) cm.$^{-1}$. The material showed a green fluoresence in sulfuric acid and the spectrum in sulfuric acid immediately after dissolving in acid had the following bands: 500 (0.28), 383 (0.19), 292 (0.60) and 239 (0.47) mμ; two hours after dissolving in acid: 485 (0.22), 390 (0.19), 330 (0.37) (shoulder), 289 (0.49) and 240 (0.55) mμ. (Values in parentheses are the optical densities for *ca.* 50γ of material in 3 cc.). Steroid IIa in tetramethylammonium hydroxide (0.066 N) showed the following *uniquely characteristic* spectrum of a 2-hydroxy-Δ⁴-3-keto steroid described by Meyer[6]: 2–3 minutes in alkali, λmax. 242 mμ (ε = 14,000); 30 minutes at 60°, λmax. 231 mμ (22,600), λinflection 252–256 mμ (7,560), λminimum 290 mμ (920), λmax. 355 mμ (2,300); after acidification, λmax. 259, λinflection 290 mμ.

Synthetic II was prepared by treating I-acetate with lead tetraacetate according to Sondheimer, *et al.*[5] Since no crystalline 2-acetoxycortisol could be obtained by chromatography,[7] the reaction mixture was hydrolyzed with $KHCO_3$[5] and chromatographed twice on paper. Crystalline 2α-hydroxycortisol, m.p. 188–192°, was isolated as the major $C_{21}O_6$ reaction product which was identical (infrared, spectrum in sulfuric acid, spectrum in alkali and mobility on paper) with steroid IIa. The structure 2α-hydroxycortisol was assigned to the synthetic material because of its method of preparation which is known to lead to 2-acetoxysteroids.[5,8,9] In addition, II is different from the previously described more polar 6β-hydroxycortisol,[2] the other possible product of the reaction at an allylic position. (The 6α-hydroxy structure can be excluded owing to the fact that it would be of slower mobility and exhibit an entirely different spectrum in alkali[6].) The 2α-hydroxy configuration follows from the fact that hydrolysis with

(1) The work was supported in part by Research Grant No. NSF-G664, National Science Foundation.
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(6) A. S. Meyer, *J. Org. Chem.*, **20**, 1240 (1955).
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$KHCO_3$ under the conditions used[5,10] leads to the more stable 2α-hydroxy epimer.

(10) R. L. Clarke, K. Dobriner, A. Mooradian and C. Martini, THIS JOURNAL, **77**, 661 (1955).

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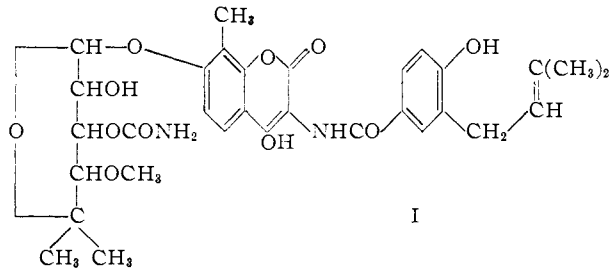
NOVOBIOCIN. II. STRUCTURE OF NOVOBIOCIN

Sir:

Formula I represents the structure of novobiocin (I).

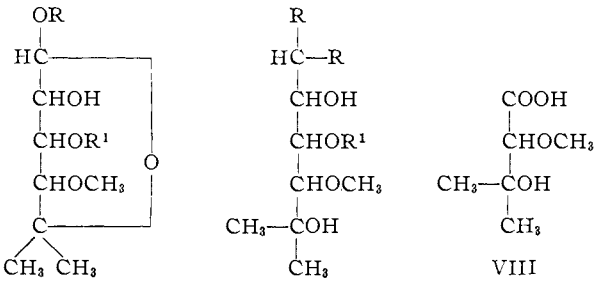


I

Review statements on the isolation of novobiocin in three laboratories have been made,[1] and initial structural studies have been described.[1,2] A recent communication[3] presents a partial structure of novobiocin which is in agreement with structure I.

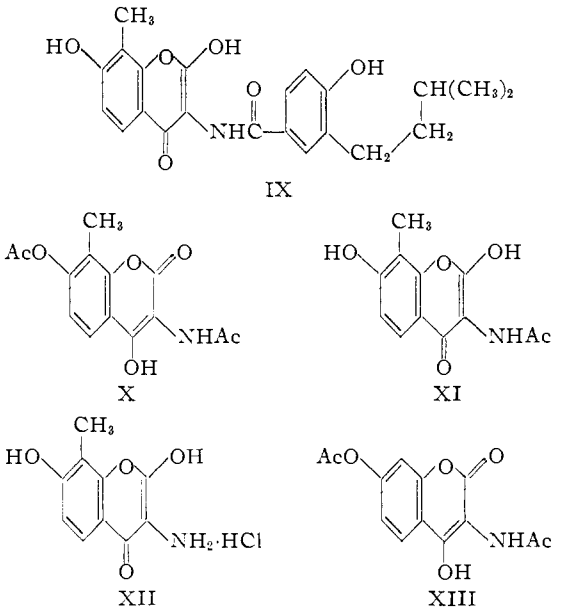
Cleavage of novobiocin with methanolic hydrogen chloride yielded methyl 3-carbamyl-4-methylnovobioside,[1] $C_{10}H_{19}NO_6$ (II), m.p. 191–192°. This glycoside did not react with sodium periodate. Hydrolysis of II with dilute hydrochloric acid gave 3-carbamyl-4-methylnovobiose (III) (Calcd. for $C_9H_{17}NO_6$: N, 5.96. Found: N, 5.98), which reacted with one mole of sodium periodate. Thus, an hydroxyl group is present on the carbon adjacent to the glycosidic carbon. Alkaline hydrolysis of II formed ammonia, carbon dioxide and methyl 4-methylnovobioside (IV) (Calcd. for $C_9H_{18}O_5$: C, 52.41; H, 8.80. Found: C, 52.70; H, 8.31). These products show the presence of a urethane group in II, and the infrared spectrum is consistent with this formulation. Periodate oxidation of IV (one mole of periodate consumed) yielded glyoxal.

Reaction of methyl 3-carbamyl-4-methylnovobioside (II) with ethyl mercaptan and hydrogen chloride gave the mercaptal (V), m.p. 143–145° (Calcd. for $C_{13}H_{27}NO_5S_2$: C, 45.72; H, 7.97; S, 18.78. Found: C, 45.04; H, 7.22; S, 19.15). Treatment of V with Raney nickel gave VI, m.p. 117–118°, (Calcd. for $C_9H_{19}NO_5$: C, 48.55; H, 8.66; N, 6.33. Found: C, 49.10; H, 8.00; N,



II, R = CH_3; R^1 = $CONH_2$
III, R = H; R^1 = $CONH_2$
IV, R = CH_3; R^1 = H
V, R = SEt; R^1 = $CONH_2$
VI, R = H; R^1 = $CONH_2$
VII, R = R^1 = H
VIII

6.68) which did not react with sodium periodate. Alkaline hydrolysis of VI yielded VII, which reacted with one mole of sodium periodate giving a mole of acetaldehyde. Periodate oxidation of VII followed by bromine oxidation of the reaction product yielded (−)-α-methoxy-β-hydroxyisovaleric acid (VIII), which formed a crystalline N,N′-dibenzylethylenediamine salt, m.p. 119–120° (Calcd. for $C_{28}H_{41}N_2O_8$: C, 62.67; H, 8.27; N, 5.22. Found: C, 62.93; H, 8.07; N, 4.99). The infrared spectrum of this salt was identical with that of its optical antipode, which was synthesized from (−)-α,β-dihydroxyisovaleric acid.[4]



IX

X

XI

XII

XIII

Cleavage of dihydronovobiocin[1,2] with hydrochloric acid in methanol gave dihydronovobiocic acid (IX) (Calcd. for $C_{22}H_{23}NO_6$: C, 66.49; H, 5.83; N, 3.53; O, 24.2. Found: C, 66.66; H, 5.68; N, 3.84; O, 23.4). Treatment of IX with hydrogen bromide–acetic acid–acetic anhydride gave X, pK_a = 4.9, (Calcd. for $C_{14}H_{13}NO_6$: C, 57.72; H, 4.50; N, 4.81; CH_3CO, 29.6. Found: C, 57.72; H, 4.11; N, 4.90; CH_3CO, 24.8). Alkaline deacetylation of X gave XI, pK_a = 5.3 and 11.1 (Calcd. for $C_{12}H_{11}NO_5$: C, 57.83; H, 4.45;

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