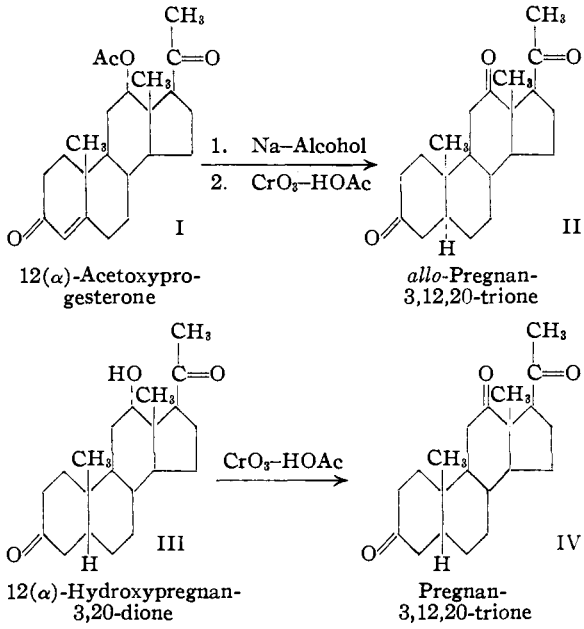

12(α)-Acetoxyprogesterone — *allo*-Pregnan-3,12,20-trione

12(α)-Hydroxypregnan-3,20-dione — Pregnan-3,12,20-trione

to lead to the *allo*-configuration at C-5. Nevertheless, we have prepared the corresponding isomer, pregnan-3,12,20-trione (IV), by the mild oxidation of an authentic sample of 12(α)-hydroxypregnan-3,20-dione (III). It had the following properties: m.p. 204–206°, $[\alpha]^{26}D$ +181°, $[\alpha]^{26}_{5461}$ +225° (chloroform). *Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.3; H, 9.2. Found: C, 76.0; H, 9.1. Reichstein and von Arx[5] report for pregnan-3,12,20-trione: m.p. 201–202°; $[\alpha]^{17}D$ +182 ± 7, $[\alpha]^{17}_{5461}$ +219 ± 8 (ethanol). A mixture of IV with II showed a melting point depression of 36°. The melting point of each of these compounds was depressed 10–20° by the trione from hecogenin.

Since the properties of *allo*-pregnan-3,12,20-trione (II) are different from those of the samples derived from hecogenin and botogenin, some doubt must be entertained as to the structures of the degradation products from both of these sapogenins.

We thank Parke, Davis and Company for their help.

(5) Reichstein and von Arx, *Helv. Chim. Acta*, **23**, 747 (1940).

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RECEIVED OCTOBER 10, 1949

SYNTHESES IN THE DIRECTION OF MORPHINE. I. 7-METHOXY- AND 7,8-DIMETHOXY-2-TETRALONE.

Sir:

We wish to report the synthesis of 7,8-dimethoxy-2-tetralone, which may serve as a useful intermediate for elaboration in the direction of morphine and certain of its degradation products,[1] and may open a way for the preparation of physiologically active substances oxygenated at points corresponding to the 3 and 4 positions in morphine. 7-Methoxy-2-tetralone may serve in the syntheses of substances similarly substituted in the 3 position; and is of particular interest in view of the recent report that 3-hydroxymorphinane is a powerful analgesic surpassing morphine in clinical tests[2].

1,2,7-Trimethoxynaphthalene,[3] m.p. 38.5–39.5°, b.p. 133° at 1 mm. (picrate[3], m.p. 113°), gave by reduction[4] with sodium and alcohol, the crystalline ketone, m.p. 76° (*anal.* calcd. for $C_{10}H_8O(OCH_3)_2$: OCH_3, 30.1. Found: OCH_3, 29.5, 29.3), characterized as the semicarbazone, m.p. 191–191.5°, and the 2,4-dinitrophenylhydrazone, m.p. 167° dec. (*anal.* calcd. for $C_{18}H_{18}O_8N_4$: C, 56.0; H, 4.7; N, 14.5. Found: C, 55.7; H, 4.6; N, 15.0, 14.8). The structure of the ketone was shown by oxidation, with alkaline permanganate, to hemipinic acid, identified by its m.p.[5] (177–179°) and by the m.p.[6] (166–167°) and characteristic fluorescence[6] of the pure anhydride.

2,7-Dimethoxynaphthalene similarly[4] gave on reduction 7-methoxy-2-tetralone, m.p. 27–28°, b.p. 124–126° (1.5 mm.); semicarbazone, m.p. 174–176° (*anal.* calcd. for $C_{12}H_{15}O_2N_3$: C, 61.8; H, 6.5. Found: C, 62.1, 62.1; H, 6.4, 6.4); 2,4-dinitrophenylhydrazone m.p. 177–181° (*anal.* calcd. for $C_{17}H_{16}N_4O_5$: C, 57.3; H, 4.5. Found: C, 57.2, 57.5; H, 4.4, 4.6).

(2) Schnider and Grussner, *Helv. Chim. Acta*, **32**, 821 (1939).
(3) Chakravarti and Pasupati, *J. Chem. Soc.*, 1859 (1937).
(4) Cornforth, Cornforth and Robinson, *ibid.*, 689 (1942).
(5) Perkin, *ibid.*, **109**, 922 (1916).
(6) Dobbie and Lauder, *ibid.*, **67**, 19 (1895).

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RECEIVED OCTOBER 19, 1949

DEGRADATION OF GLYCOGEN TO ISOMALTOSE

Sir:

Methylation studies[1] have indicated that the glycogen molecule has a highly ramified structure composed of α-D-glucopyranosyl units joined 1,4 with branching at C6 on one out of twelve units. As additional evidence in support of this structure we report the isolation of crystalline 6-α-D-glucopyranosyl-β-D-glucopyranose octaacetate (β-D-isomaltose octaacetate)[2] from an acetylated acid hydrolysate of glycogen.

Animal (rabbit liver) glycogen (5.00 g., $[\alpha]^{25}D$ +200°, *c* 0.92, water) in 2% concentration was hydrolyzed at 100° in 0.05 *N* sulfuric acid for nine hours (degree of hydrolysis *ca.* 75%). After acid neutralization with barium carbonate and ion removal with exchange resins (Amberlite IR-100 and IR-4), the amorphous solid obtained on solvent removal was acetylated with hot acetic anhydride and sodium acetate. The resultant sugar acetate mixture (6.08 g.) was chromatographed[2] on Magnesol–Celite under such developmental conditions that monosaccharides were removed from the column. β-D-Glucose pentaacetate was identified,

(1) Fieser and Holmes, THIS JOURNAL, **60**, 2548 (1938); **58**, 2319 (1936); Cahn, *J. Chem. Soc.*, 2565 (1926).

(1) W. N. Haworth and E. G. W. Percival, *J. Chem. Soc.*, 2277 (1931); W. N. Haworth, E. L. Hirst and F. Smith, *ibid.*, 1914 (1939).

(2) M. L. Wolfrom, L. W. Georges and I. L. Miller, THIS JOURNAL, **69**, 473 (1947); **71**, 125 (1949).

by melting point and rotation, in the effluent. The material from the lowest zone consisted of β-D-maltose octaacetate (m. p. 158–160°, unchanged on admixture with an authentic specimen; $[\alpha]^{25}D$ +62.5°, *c* 1.1, chloroform). The material from the next higher zone was rechromatographed in the same manner, and the eluent from the lower zone which crystallized from ethanol was identified as β-D-isomaltose octaacetate (m. p. 144–145°, unchanged on admixture with an authentic specimen; $[\alpha]^{26}D$ +98°, *c* 1.0, chloroform); yield 92 mg.

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RECEIVED OCTOBER 8, 1949

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ERYTHEIN AND APOERYTHEIN AND THEIR RELATION TO THE ANTIPERNICIOUS ANEMIA PRINCIPLE

Sir:

Normal gastric juice has been found to contain a non-dialyzable, heat labile substance which combines, apparently stoichiometrically, with erythrotin,[1] (vitamin B_{12})[2] to form a complex (erythein) which is non-dialyzable and not dissociated by dialysis. Erythrotin in this combination is not available to microörganisms (*Escherichia coli*, *Lactobacillus lactis Dorner*, *Lactobacillus leichmannii*), but is released by heat, much as biotin is released from avidin, whereupon it is again microbiologically active. Heated gastric juice contains no principle capable of combining with erythrotin.

Quantitative determination of heat labile principle (apoerythein) is readily performed by measuring in an erythrotin assay (*Escherichia coli*)[1] the inhibition of growth resulting when aliquots of the juice are added (unheated) to cultures containing just sufficient erythrotin to elicit a maximum response. The erythrotin combining capacities (millimicrograms of erythrotin per ml. of secretion) of samples of gastric juice from normal and anemic subjects were found to be respectively, 20, 60[3], 60, 15; and 5, <5,[3] <1, <1 >15.

Commercial preparations of hog gastric mucosa made for therapeutic use have been found to be rich in a principle which appears on the basis of chemical and biological properties to be analogous to the apoerythein in gastric juice. Other biological materials tested, including commercial pepsins, contain very little or none of the active substance. Less than 2000 parts by weight of a concentrate prepared from hog gastric mucosa completely counteracted consistently the microbiological growth stimulation of one part of erythrotin.

For preparative purposes hog gastric mucosa has been used, and the principle can be precipitated from an aqueous extract by alcohol, acetone or ammonium sulfate (80% saturation). The principle is highly selective in its action and inactivates erythrotin but does not diminish the biological action of the end-products of erythrotin-catalyzed processes which can substitute for this vitamin in microbiological assays—methionine (*Escherichia coli* test)[1] and desoxyribosides (*Lactobacilli* tests).[4]

The complex formed when erythrotin combines with apoerythein decomposes upon heating (120° fifteen minutes) into erythrotin (or a compound which cannot be distinguished from it biologically or chromatographically), and a residue no longer possessing the ability to bind erythrotin. In combined form erythrotin is not as susceptible to destruction by alkaline or oxidative treatments which inactivate the unbound vitamin, since heat liberation following such treatment of the complex yields the original erythrotin activity.

These experiments point to the probability that apoerythein is the intrinsic factor of Castle or an important component thereof. Clinical trials are now in progress to test this conclusion.

We are deeply indebted to Dr. William Shive for generous supplies of erythrotin before vitamin B_{12} was available and for prepublication disclosures concerning erythrotin tests, and to Dr. Edward Campbell, Eli Lilly and Company, who furnished biological preparations and gastric samples.

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(1) W. Shive, *Ann. N. Y. Acad. Sci.*, in press.
(2) E. L. Rickes, *et al.*, *Science*, **107**, 396 (1948).
(3) Pooled samples from at least three subjects.
(4) W. Shive, J. M. Macow and R. E. Eakin, THIS JOURNAL, **70**, 2614 (1948).