L(+)2-TROPINONE

Sir:

We wish to report the synthesis of L(+)2-tropinone, a key degradation product of the alkaloid dioscorine.[1] L-Cocaine hydrochloride[2] was hydrolyzed to ecgonine, which in turn was treated with phosphorus oxychloride to produce the unisolated acid chloride of anhydroecgonine.[3] Cold aqueous ammonia converted the chloride to L(−)-anhydroecgonine amide, m.p. 142.5–145°, $[\alpha]^{25}D$ −51.2° (1% in H_2O).

(*Anal.* Calcd. for $C_9H_{14}N_2O$: C, 65.03; H, 8.49; N, 16.86. Found: C, 65.27; H, 8.35; N, 16.66).[4] Treatment of the amide with sodium hypochlorite in aqueous methanol and then acid hydrolysis furnished L(+)2-tropinone, b.p. 60–61° (0.55 mm.), m.p. *ca.* 27°, $[\alpha]^{30}D$ +23.0° (1.6% in H_2O) (*anal.* Calcd. for $C_8H_{13}NO$: C, 69.03; H, 9.41; N, 10.06. Found: C, 69.36; H, 9.46; N, 9.89).

The infrared spectrum showed carbonyl absorption at 5.82 μ ($CHCl_3$ solution and liquid film). The methiodide, m.p. >330°, (*anal.* Calcd. for $C_9H_{16}INO$: I, 45.14. Found: I, 45.28) absorbed at 5.76 μ (Nujol). The hydrobromide, m.p. 266–266.5°, reconvertible to the parent base, crystallized from methanol as a methanolate which was devoid of carbonyl absorption (KBr disc) and was therefore formulated as the hemiketal (*anal.* Calcd. for $C_8H_{13}NO\cdot HBr\cdot CH_4O$: C, 42.86; H, 7.19; Br, 31.69; CH_3O, 12.31. Found: C, 42.78; H, 6.84; Br, 32.20; CH_3O, 11.78).[5]

Apparently the proximity of the positively charged nitrogen in the 2-tropinone salts to the trigonal carbon is responsible for both the shift of ketonic absorption to lower wave lengths and the ready solvation of the carbonyl group.

Reduction of 2-tropinone with lithium aluminum hydride gave mainly 2α-tropanol, m.p. 73–76° (*anal.* Calcd. for $C_8H_{15}NO$: C, 68.04; H, 10.71; N, 9.92. Found: C, 67.61; H, 10.63; N, 9.88), (hydrochloride, m.p. 268–269.5° (dec.) (*anal.* Calcd. for $C_8H_{16}ClNO$: N, 7.88. Found: N, 7.81), and a small amount of the liquid 2β-tropanol isolated as the hydrochloride, m.p. 326–328° (dec.) (*anal.* Found: N, 7.70). Reduction of 2-tropinone by a slight modification of Dev's[6] sodium–propanol-2 method afforded in about 55% yield, 2β-tropanol substantially free of the alpha isomer (vapor phase chromatography and infrared spectrum). Since the intensity of the broad hydroxyl band in the spectrum of the solid isomer was concentration dependent and the intensity of the sharp hydroxyl band of the liquid isomer did not change on dilution, the latter alcohol shows intramolecular hydrogen bonding and must be 2β-tropanol. This is a case wherein metal–alcohol reduction of an unhindered ketone affords predominantly if not exclusively the axial alcohol.

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RECEIVED OCTOBER 4, 1958

(1) D. E. Ayer, G. Büchi, P. Reynolds Warnhoff and Dwain M. White, THIS JOURNAL, **80**, 6146 (1958).

(2) E. Hardegger and H. Ott, *Helv. Chim. Acta*, **38**, 312 (1955).

(3) A. Einhorn, *Ber.*, **20**, 1221 (1887).

(4) Analyses and spectral determinations were carried out under the supervision of M. E. Auerbach and F. C. Nachod, respectively.

(5) Earlier, R. E. Lyle, *et al.*, *J. Org. Chem.*, in press, found that 1-methyl-3-piperidone behaved similarly, *i.e.*, the base absorbed at 5.82 μ (liquid film) and the corresponding hydrochloride formed a stable hydrate that was transparent in the carbonyl region in the infrared. We wish to thank Dr. Lyle for supplying this information in advance of publication.

(6) S. Dev, *J. Ind. Chem. Soc.*, **33**, 769 (1956).

HYDANTOIN-5-PROPIONIC ACID: A NEW URINARY METABOLITE OF UROCANIC ACID

Sir:

Some unidentified acidic metabolites have been found in urine after administration of radioactive histidine and urocanic acid (I) to mammals.[1] They could not be accounted for as intermediates in the known catabolic pathway of I which proceeds through breakage of the ring to formiminoglutamic acid (II) and finally glutamic acid.[2] Alternate pathways have been demonstrated in bacteria[3] and have been induced *in vitro* by the addition of oxidants (dichlorophenolindophenol (III) or ferricyanide) to I in the presence of purified liver urocanase.[4] It was suggested to us by Dr. B. Witkop that the hydantoin structure could account for the marked acidity of these unknown compounds.

Ring labelled (2-C^{14}) I was made enzymatically from L-histidine (Nuclear-Chicago),[5] and 4.8 μc. (9.3 mg.) were injected intraperitoneally into a 200-g. white male rat. Urine was collected under toluene; 10% of the injected radioactivity was excreted during the first 12 hours.

Urine was chromatographed directly on Dowex-1×8-acetate (100–200 mesh, 50 drops/tube). Samples were collected automatically, dried, and counted on a Packard Tri-Carb liquid scintillation counter after the addition of hyamine and phosphor.[6] Figure 1 shows the elution pattern. Un-

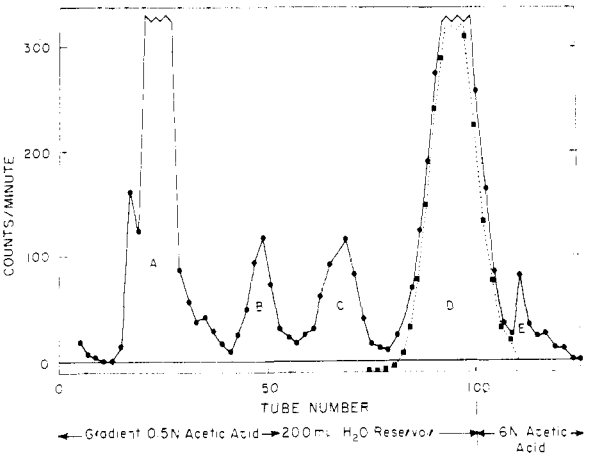

Fig. 1.—Urinary metabolites of 2-C^{14} urocanic acid in the rat.

(1) M. Kraml and L. P. Bouthillier, *Canad. J. Biochem. Physiol.*, **34**, 783 (1956); G. Wolf, P. L. Wu and W. W. Heck, *J. Biol. Chem.*, **222**, 159 (1956).

(2) H. Tabor, *Pharmacol. Rev.*, **6**, 299 (1954).

(3) K. Ichihara, Y. Sakamoto, H. Satani, N. Okada, S. Kakiuchi, T. Koizumi and S. Ota, *J. Biochem. (Japan)*, **43**, 797 (1956).

(4) A. Miller and H. Waelsch, *J. Biol. Chem.*, **228**, 365 (1957); R. H. Feinberg and D. M. Greenberg, *Nature*, **181**, 897 (1958).

(5) A. H. Mehler, H. Tabor and O. Hayaishi, *Biochem. Preps.*, **4**, 50 (1955).

(6) J. M. Passmann, N. S. Radin and J. A. D. Cooper, *Anal. Chem.*, **28**, 484 (1956).

changed I (peak A) was identified by recrystallization with carrier from water to constant specific activity. The radioactivity in peak B could be volatilized by treatment with ammonia and acidification with formic acid, identifying it indirectly as II.[7] Peak D was shown to be hydantoin-5-propionic acid (IV) by recrystallization with carrier to constant specific activity from both ethanol–benzene and water and co-chromatography in six solvents with the synthetic compound.[8] Synthetic IV (Found: C, 41.69; H, 4.60; N, 16.29) prepared from L-glutamic acid[9] was eluted in the identical position as peak D. The dotted line in Fig. 1 is synthetic radioactive IV. Peaks A–D have 72, 1.1, 1.5 and 8.5% of the urinary radioactivity, respectively.

Incubation of I with rat liver slices forms small amounts of IV in the presence or absence of III; radioactive IV is present in monkey and human urine after intravenous C^{14} histidine.

The biochemical steps from I to IV have not yet been elucidated.

Acknowledgment.—The authors are grateful to Dr. Herbert Tabor for his helpful criticism.

(7) B. A. Borek and H. Waelsch, *J. Biol. Chem.*, **205**, 459 (1953).

(8) Synthetic IV sprayed with 0.1 *M* $AgNO_3$: 0.1 *M* NH_4OH (1:1) is white against a brown background. Radioautograph spots matched the outline and position of stained spots exactly. R_F values for benzene: 1-butanol:methanol:H_2O (1:1:2:1), 2-butanol:formic acid:H_2O (19:2:6), acetic acid:1-butanol:ethyl acetate:H_2O (1:1:1:1), ethanol:ether:H_2O:7.4 *N* NH_3 (4:5:1:0.1), 1-propanol:1 *N* acetic acid (3:1), 2-propanol:NH_3:H_2O (8:1:1) were 0.64, 0.67, 0.68, 0.13, 0.69, 0.07, respectively.

(9) H. D. Dakin, *Biochem. J.*, **13**, 398 (1919).

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MICROBIOLOGICAL TRANSFORMATIONS. III.[1] THE HYDROXYLATION OF STEROIDS AT C-9

Sir:

We wish to report the microbiological preparation and proof of structure of 9α-hydroxy-4-androstene-3,17-dione. This compound and the method used to establish its structure may help resolve the difficulties previously experienced in the formulation of 8- or 9-hydroxysteroids.[2]

Fermentation of 4-androstene-3,17-dione, by the methods previously described,[3] with a species of Nocardia (A20-10) isolated from soil produced 9,10-seco-3-hydroxy-1,3,5(10)-androstatriene-9,17-dione[1] and a monohydroxy-4-androstene-3,17-dione (I), m.p. 222–223.5°; $\lambda_{max.}^{methanol}$ 241 mμ (ε 16,100); $[\alpha]D$ +181.7° ($CHCl_3$); λ_{max}^{KBr} 2.92 μ (—OH), 5.76 μ (17 C=O), 6.02 μ and 6.19 μ (3 C=O, Δ^4); (found: C, 75.21; H, 8.68). The hydroxy-4-androstene-3,17-dione (I) was recovered unchanged when treated with pyridine and acetic anhydride. Fermentation of I with a species of Arthobacter (B 20-178) that converts 4-androstene-3,17-dione to 1,4-androstadiene-3,17-dione in excellent yield, gave 9,10-seco-3-hydroxy-1,3,5(10)-androstatriene-9,17-dione. The latter compound was purified as its acetate, m.p. 143.5–146°, which proved to be identical in all respects (m.p., mixed m.p., and infrared) with the 9,10-seco-3-acetoxy-1,3,5(10)-androstatriene-9,17-dione reported previously.[1] Thus, the positions of the three oxygen atoms in I were established. The 9α-configuration was assigned to the new hydroxyl group because of its molecular rotatory contribution ($\Delta M_D^{OH-H} = -18$)[4] and because of the recent evidence that microbiologically introduced hydroxyl groups have the same configuration as the hydrogens replaced.[5]

In the aromatization-degradation of 4-androstene-3,17-dione it seems probable that this species of Nocardia[6] first hydroxylates at C-9 then introduces the Δ^1-double bond. This is just the opposite sequence originally found with *Pseudomonas*.[1] A paper chromatographic study of the fermentation of 9α-hydroxy-4-androstene-3,17-dione with *Pseudomonas* showed the formation of, at most, only trace quantities of phenolic material. With Pseudomonas the sequence in which the reactions occur seems to be limited.

(1) Previous paper: R. M. Dodson and R. D. Muir, THIS JOURNAL, **80**, 5004 (1958). The numbers assigned to the organisms are our laboratory designations.

(2) (a) S. H. Eppstein, P. D. Meister, D. H. Peterson, H. C. Murray, H. M. Leigh Osborn, A. Weintraub, L. M. Reineke and R. C. Meeks, *ibid.*, **80**, 3382 (1958); (b) D. Stone, M. Hayano, R. I. Dorfman, O. Hechter, C. R. Robinson and C. Djerassi, *ibid.*, **77**, 3926 (1955).

(3) D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister and H. M. Leigh, *ibid.*, **74**, 5933 (1952).

(4) The molecular rotatory contribution of the 9α-hydroxyl group in 3β-acetoxyergostan-9α-ol was −31. A. S. Hallsworth and H. B. Henbest, *J. Chem. Soc.*, 4604 (1957). The molecular rotatory contribution of the new (8 or 9) hydroxyl group in the steroids hydroxylated with *Helicostylum piriforme*, *Mucor parasiticus*, *Mucor griseocyanus* and *Neurospora crassa* (Ref. 2) indicates the probability of 9α, rather than 8β, hydroxylation. See: S. H. Eppstein, P. D. Meister, H. C. Murray and D. H. Peterson, "Vitamins and Hormones," Vol. XIV, 388 (1956), Academic Press, Inc., New York, N. Y. However, the specific rotation of the previously described 8β (or 9α)-hydroxy-4-androstene-3,17-dione,[2a] m.p. 214–217°, $[\alpha]D$ +165° ($CHCl_3$), obtained via the hydroxylation of 11-deoxycortisol with *H. Piriforme*, does not agree with ours.

(5) (a) M. Hayano, M. Gut, R. I. Dorfman, O. K. Sebek and D. H. Peterson, THIS JOURNAL, **80**, 2336 (1958); (b) E. J. Corey, G. A. Gregoriou and D. H. Peterson, *ibid.*, **80**, 2338 (1958).

(6) We have isolated another strain of Nocardia (A20-9) which apparently follows the alternate sequence.

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FREE RADICAL ADDITION OF CYCLOPENTANE AND CYCLOHEXANE TO FORMALDEHYDE

Sir:

Although alkyl free radical attack upon an aldehyde is most likely to result in hydrogen abstraction[1]

$$R' + RCHO \longrightarrow R'H + \dot{R}C{-}O \qquad (1)$$

addition to the carbonyl group also has been demonstrated.[2]

$$R + RCHO \longrightarrow R\dot{C}HR \longrightarrow RCHOHR \qquad (2)$$

Consideration of reaction (2) leads to the conclusion that it should be possible to bring about

(1) For summary, see Steacie, "Atomic and Free Radical Reactions," 2nd Ed., Reinhold Publishing Corp., New York, N. Y., 1954.

(2) F. F. Rust, F. H. Seubold and W. E. Vaughan, THIS JOURNAL, **70**, 4253 (1948).