TABLE II

SHIFT IN *p*H OPTIMUM WITH DECREASING ACTIVITY

Time after grinding leaves, hours	*p*H optimum	$Q_{O_2}^{ch}$
1–2	≥8.70	2150
5–6	8.40	1670
10–11	8.25	1370
34–35	flat response	590
53–54	6.85	250

It should be pointed out that the extremely high initial *p*H optimum (≥8.7) was associated with exceptional stability of the chloroplasts, for it was found with only three different chard chloroplast preparations made during one week of July, 1956. Usually the *p*H optimum of highly active chloroplasts ($Q_{O_2}^{ch}$ 1800 or higher) is between 8.0 and 8.5, and decreases with decreasing activity. With a few exceptions the optimum does not depend on the species of plant used as the source of chloroplasts. The foregoing illustrates the importance of expressing the activity in absolute terms ($Q_{O_2}^{ch}$) when determining the *p*H optimum for any preparation.

When these results are compared with those obtained by others, it is found that in all cases low *p*H optima are associated with low activity,[2,5,6] or with prolonged preincubation of the chloroplasts in alkaline medium[1,3,4,7] which is known to inactivate them,[9] or with both.

In contrast to the results obtained when using high intensity light, the *p*H response for the Hill reaction in light of low intensity (light limited reaction) proves to be flat from *p*H 6.35 to 8.40.

A more complete description of the results reported here and earlier[9] will be published elsewhere.

This work was done while the author was a postdoctoral fellow of the National Foundation for Infantile Paralysis at the School of Biochemistry, Cambridge, England.

The author wishes to express his appreciation to Dr. R. Hill for his help and advice during the course of this investigation.

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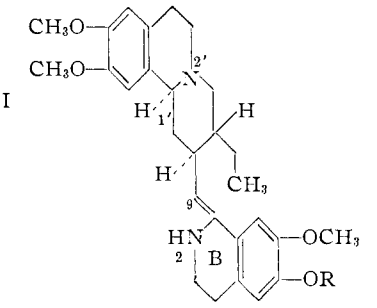
RECEIVED JUNE 5, 1957

STEREOCHEMISTRY OF THE IPECAC ALKALOIDS

Sir:

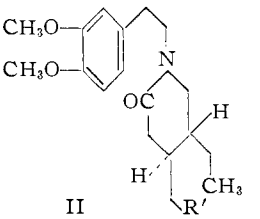
Despite the intensive and thorough investigations carried out on the alkaloids of Ipecac root,[1] including the reported total synthesis[2] of the best-known member of the family, *l*-emetine, there has not been available any direct evidence bearing on the stereochemical aspects of these important bases. Largely through the correlation of an intermediate used in the total synthesis[2] with a reference compound of proved stereochemistry, it is now possible to propose the stereoformula I[3] for psychotrine (R = H), O-methylpsychotrine (R = CH_3), cephaëline (R = H, 1,9-double bond saturated), emetamine (R = CH_3, ring B aromatic) and emetine (R = CH_3, 1,9-double bond saturated), all members of the same stereochemical family.[1]

I

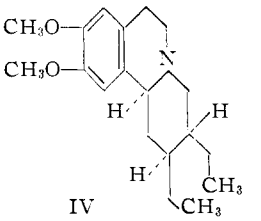


Alkylation of 3,4-dimethoxy-β-phenethylamine with ethyl *dl-threo*-3,4-diethyl-5-bromovalerate[4] yields the reference compound, the lactam (II, R′ = CH_3) of *dl*-N-(3,4-dimethoxy-β-phenethyl)-*threo*-3,4-diethyl-5-aminovaleric acid, b.p. 128–130° (0.1 mm) (Found: C, 71.15; H, 8.92). Phosphorus oxychloride cyclization to the unisolated 3,4-dihydroisoquinolinium cation (III), followed by saturation of the carbon–nitrogen double bond (*vide infra*) provided the tetrahydroisoquinoline (IV) (hydrochloride, m.p. 247.5–248.5°. Found: C, 66.70; H, 8.57). This same[5] pair of substances was obtained from the emetine synthesis intermediate II (R′ = $COOC_2H_5$)[2] by (i) controlled reduction with lithium borohydride, which afforded the lactam alcohol II (R′ = CH_2OH), m.p. 116–117° (Found: C, 68.20; H, 8.65), followed by (ii) conversion to the tosylate and thence to the isothiouronium salt, which, without purification, was reductively desulfurized by treatment with Raney yielding *dl*-II (R′ = CH_3); transformation to the *dl*-base IV confirmed the identity of this intermediate.

II



IV



Catalytic hydrogenation of the 3,4-dihydroisoquinolinium salt III to the tetrahydro-base IV involves axial attachment of hydrogen, since sodium-ethanol reduction also yields IV. Therefore, emetine also appears to possess the more stable configuration at C-1′, as depicted in formula I, since the synthetic *dl*-alkaloid is obtained[2] by catalytic reduction of the di-salt (V) of *dl*-I (R = CH_3) $\Delta^{1'(2')}$ and $\Delta^{1(2)}$ rather than $\Delta^{1(9)}$ [6]; this view is supported by the observation that the same reduction

(1) M.-M. Janot, R. H. F. Manske and H. L. Holmes, "The Alkaloids," Vol. III, Academic Press, Inc., New York, 1953, pp. 363–394.

(2) R. P. Evstigneeva, R. S. Livshits, M. S. Bainova, L. I. Zakharkin and N. A. Preobrazhenskii, *J. Gen. Chem.*, **22**, 1467 (1952).

(3) Formula I bears no implication as to absolute configuration.

(4) E. E. van Tamelen, P. E. Aldrich and T. J. Katz, *Chem. and Ind.*, 793 (1956).

(5) On the basis of appropriate infrared spectral comparisons and a m.m.p. on the hydrochloride salts of IV.

(6) Our repetition of the Russian work (ref. 2) indicates that isoemetine (epimeric with emetine at C-1) is also produced in this step.

product[6] is provided by the action of lithium aluminum hydride on this intermediate V.[7]

Acknowledgment.—The authors are grateful to Dr. H. T. Openshaw for a sample of isoemetine hydrobromide. Financial support was provided by the research committee of the Graduate School.

(7) It is pertinent that in the case of the Δ^3-dehydroyohimbane (D-E *trans*) and similar (*cf.* ref. 4) systems, catalytic reduction also affords the more stable 3,15,20-*cis-trans* product (E. E. van Tamelen, M. Shamma and P. E. Aldrich, THIS JOURNAL, **78**, 4628 (1956).

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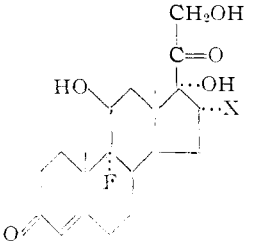
OXIDATION OF STEROIDS BY MICROORGANISMS. IV. 16α-HYDROXYLATION OF 9α-FLUOROHYDROCORTISONE AND 9α-FLUOROPREDNISOLONE BY *Streptomyces roseochromogenus*

Sir:

The introduction of a 16α-hydroxyl group into 9α-fluorohydrocortisone (I) and 9α-fluoroprednisolone (II) has been shown by Bernstein, *et al.*,[1] to result in complete suppression of the salt-retaining properties of these steroids without appreciably impairing their glucocorticoid activity. Moreover, preliminary studies in man[2] have demonstrated the anti-arthritic activity of 9α-fluoro-16α-hydroxyprednisolone (triamcinolone) and have confirmed its lack of salt-retaining activity. An efficient synthesis of this complex steroid is therefore of considerable practical interest.



I, X = H
II, X = H; $\Delta^{1,2}$
III, X = OH
IV, X = OH; $\Delta^{1,2}$

Applying the microbiological 16α-hydroxylation reaction first reported from this laboratory[3,4] to I and II we have succeeded in preparing 16α-hydroxy-9α-fluorohydrocortisone (III) and 16α-hydroxy-9α-fluoroprednisolone (IV) in yields of 50% and 20%, respectively.

A fermentation medium containing soybean meal (30 g.), glucose (20 g.), soybean oil (4.4 g.) and calcium carbonate (0.050 g.) in distilled water (2 l.) was distributed over 40 250-ml. erlenmeyer flasks, steam-sterilized for 30 minutes at 120° and after addition of the steroid (1 g. dissolved or suspended in 40 ml. of methanol) inoculated with vegetative growth of *Streptomyces roseochromogenus*.[5] The flasks were incubated at 25° for 4 to 7 days with rotatory mechanical shaking at 280 r.p.m. in a 2 in. radius, the mycelium filtered off and washed with water. The filtrate was extracted with methyl isobutyl ketone (three 800-ml. portions), the extract concentrated to small volume, cooled and the resulting crystals recrystallized from ethyl alcohol. The properties of III (m.p. 250–252°, $[\alpha]D$ +97° (*c* 0.99 in pyridine); λ_{max}^{ale} 238 mμ (15,000); λ_{max}^{Nujol} 2.79, 2.98, 5.82, 6.00, 6.17μ[6]; *Anal.* Found: C, 63.77; H, 7.32) and IV (m.p. 248–250°; $[\alpha]^{23}D$ +71° (c 0.35 in acetone); λ_{max}^{Nujol} 2.95, 5.85, 6.02, 6.16, 6.24, 11.26 μ; diacetate: m.p. 170–180° (with gas evolution); $[\alpha]^{23}D$ +28° (*c* 0.38 in $CHCl_3$); *Anal.* Found: C, 62.34; H, 6.74) are in harmony with those reported by Bernstein, *et al.*[1]

Oxidation of the diacetates of III and IV with chromic and sulfuric acids in acetone gave, respectively, the diacetates of 16α-hydroxy-9α-fluorocortisone, m.p. 215–217°; $[\alpha]^{23}D$ +94° (*c* 0.35 in $CHCl_3$); λ_{max}^{ale} 235 mμ (15,100); λ_{max}^{Nujol} 2.94, 5.75, 6.00, 6.19 μ; *Anal.* Found: C, 63.03; H, 6.53, and of 16α-hydroxy-9α-fluoroprednisone, m.p. 204–206°; $[\alpha]^{23}D$ +90° (c 0.41 in $CHCl_3$); λ_{max}^{ale} 235 mμ (16,200); λ_{max}^{Nujol} 2.97, 5.75, 5.99, 6.15, 6.22, 11.28 μ; *Anal.* Found: 63.13; H, 6.25.

The biosynthetic conversion of III into IV by means of *Corynebacterium simplex* has been reported.[1] We have performed this reaction in 65% yield with a strain of *Mycobacterium rhodochrous*[7] adding the steroid (0.5 g./l.) to a 24-hour old culture in a medium containing yeast extract, tryptone, pentane, glucose calcium, and incubating for five hours.

(1) S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman, and R. H. Blank, THIS JOURNAL, **78**, 5693 (1956).

(2) L. Hellman, B. Zumoff, M. K. Schwartz, T. F. Gallagher, C. A. Berntsen and R. H. Freyberg, Abstract of Papers presented at the 3rd Interim Meeting of the American Rheumatism Association, Bethesda, Md., Nov. 30, 1956.

(3) D. Perlman, E. O. Titus and J. Fried, THIS JOURNAL, **74**, 2126 (1952).

(4) J. Fried, R. W. Thoma, D. Perlman, J. E. Herz, and A. Borman, *Recent Progr. Hormone Research*, **9**, 149 (1955).

(5) Waksman Collection Number 3689, Rutgers University, New Brunswick, N. J.

(6) Occasionally a polymorphic form of III was obtained, which lacked the 2.79 μ band and showed an entirely different picture in the fingerprint region.

(7) This culture, isolated by Dr. J. O. Lampen of our laboratories, is identified as SC 2318 in our collection. With regard to its classification *cf.* R. Gordon and J. M. Mihm, *J. Bact.*, **73**, 15 (1957). Its dehydrogenating properties were discovered in these laboratories by Dr. H. Kroll.

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RECEIVED JULY 31, 1957

TOTAL SYNTHESIS OF 17-DESMETHOXYDESERPIDINE

Sir:

In a previous communication[1] we described the synthesis of the unsaturated lactone (I). We now wish to report the conversion of this intermediate to 17-desmethoxydeserpidine.

Reduction of the unsaturated lactone (I) with hydrogen and a highly active platinum catalyst for sixteen hours gave predominantly the saturated lactone (II), m.p. 267–269° (found: C, 74.81; H, 6.98; λ_{max}^{Nujol} 5.71 μ) as well as a small amount of an isomeric lactone (III), m.p. 307–309° (found: C, 74.25; H, 6.86; λ_{max}^{Nujol} 5.65 μ). When the product was allowed to remain in contact with the

(1) F. L. Weisenborn and H. E. Applegate, THIS JOURNAL, **78**, 2021 (1956).