This reaction was applied to the quantitative estimation of 1% *p*-hydroxyamphetamine found in an ophthalmic solution[2]. The concentrations (micrograms per milliliter) were calculated from the prepared standard curve. The results of these analyses are shown in Table II. The mechanism of this reaction is currently being investigated and will be reported at a later date.

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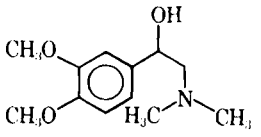
Cactus Alkaloids XIII: Isolation of (−)-Normacromerine from *Coryphantha macromeris* var. *runyonii*

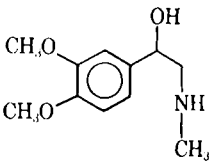
Keyphrases □ (−)-Normacromerine—isolated from *Coryphantha macromeris* □ *Coryphantha macromeris* var. *runyonii*—isolation and identification of (−)-normacromerine □ Cactus alkaloids—isolation and identification of (−)-normacromerine from *Coryphantha macromeris*

Sir:

Hodgkins *et al.* (1) isolated (−)-macromerine (I) from "Dona Ana," one of the southwestern nipple cacti [*Coryphantha macromeris* (Engelm.) Lem.], and demonstrated by animal testing that this analog of mescaline and epinephrine has potential hallucinogenic and sympatholytic activities. The same alkaloid was concurrently found by Below *et al.* (2) in *C. runyonii* Br. and R., which is now considered a variety of the former species (3). Agurell (4, 5) subsequently detected tyramine, hordenine (*N*,*N*-dimethyltyramine), *N*-methyl-3,4-dimethoxy-β-phenethylamine, and *N*-methyl-4-methoxy-β-phenethylamine as minor alkaloids of this plant. According to a recent lay publication (6), this species is now being promoted as a "natural and legal" psychedelic agent with about one-fifth the potency of peyote [*Lophophora williamsii* (Lem.) Coult.]. The present



I



II

investigation was initiated to reexamine the plant for additional alkaloids.

Using an extractor (Lloyd), 11.8 kg. of oven-dried and pulverized *C. macromeris* (Engelm.) Br. and R. var. *runyonii* L. Benson[1] was extracted with ethanol. The alkaloids were purified from the ethanol residue and resolved into phenolic and nonphenolic fractions as previously described (7, 8). TLC analysis of the nonphenolic fraction revealed a new major alkaloid, which was present in higher concentration than macromerine. The new alkaloid was visualized with dansyl chloride reagent, indicating that it was a primary or secondary amine (7). Purification by preparative TLC, using benzene–chloroform–methanol–28% ammonium hydroxide (8:6:5:1) on 1-mm. plates of silica gel PF-254, permitted crystallization of the new compound as the free base (m.p. 101–103°) and the hydrochloride (m.p. 132–133°) in approximately 0.19% yield [compared with 0.07% macromerine (2), based on the dry weight]. The isolated alkaloid appeared as a single spot upon further TLC in additional solvent systems, indicating its homogeneity.

NMR spectra of the free base in deuterochloroform showed a distribution of protons almost identical to macromerine (2); the major difference was at 2.44 δ where integration indicated three rather than six *N*-methyl protons. The UV spectrum of the hydrochloride (0.005 mg./ml. absolute methanol) showed a $\lambda_{max.}$ at 207 nm. (ε 39,000) and, respectively, smaller peaks at 229 and 277 nm., typical of 3,4-dimethoxy-β-phenethylamines (9). From these data, it was predicted that the new alkaloid was simply *N*-methyl-3,4-dimethoxy-β-hydroxy-β-phenethylamine, to which we have given the trivial name normacromerine (II). IR spectra illustrated peaks supporting this prediction. The hydrochloride salt of the natural alkaloid was levorotatory: $[\alpha]_D^{27}$ −47.5°, c 0.020 g./ml. in absolute methanol. Alterations of the extraction procedure and freeze drying, rather than heat drying, of the cacti did not affect the detection of the alkaloid in the extracts, discounting speculations that it might have been an artifact.

Racemic normacromerine hydrochloride (m.p. 115–117°) was synthesized by condensing veratrole and methylaminoacetonitrile using a Houben–Hoesch reaction followed by reduction of the aminoketone with sodium borohydride. The UV, IR, and NMR spectra of the natural (−)-normacromerine hydrochloride and the synthetic (±)-normacromerine hydrochloride were essentially identical. The primary amine, bisnormacromerine, was synthesized by similar reactions, but this

[1] Purchased from Sunderland's Cactus Garden, Alamo, TX 78516. Identification was confirmed by Dr. E. F. Anderson, Department of Biology, Whitman College, Walla Walla, WA 99362. Representative plants are being maintained as greenhouse specimens.

compound was not detectable in the plant extracts using TLC.

This communication is the first report of the occurrence of this simple β-phenethylamine in nature. Considering its relatively high concentration and structural similarity to other physiologically active alkaloids, normacromerine may be at least partially responsible for the psychotropic effects attributed to the plant.

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Facile Differential UV Determination of Steroids with Conjugated Ketone Chromophores *via* Lithium Borohydride Reduction

Keyphrases ☐ Steroids, conjugated keto—identification *via* lithium borohydride reduction in tetrahydrofuran, differential UV determination ☐ Lithium borohydride reduction in tetrahydrofuran—determination of conjugated ketosteroids, differential UV spectrophotometry ☐ UV spectrophotometry, differential—determination of conjugated ketosteroids *via* lithium borohydride reduction

Sir:

Many corticosteroids, progestins, and androgenic steroid drugs have 3-keto functions conjugated with double bonds. The ketone moiety in these conjugated systems is reduced to hydroxyl by metal borohydrides, thereby abolishing the conjugation and the strong UV absorption which result from it. Several workers (1–5) exploited this reaction in the selective analysis of steroids. Görög (5) described an elegant differential UV spectrophotometric method for conjugated 3-ketosteroids, measuring the absorbance of an aliquot of steroid solution added to previously decomposed sodium borohydride against a reference solution prepared by reducing a similar aliquot with the reagent. In this way, interference from other types of steroids and from many formulation components which may be present in the sample is obviated. According to Görög (5), steroids with the 4-en-3-one function are reduced in 15 min. at room temperature using sodium borohydride in methanol; however, those with the 1,4-dien-3-one chromophore require heating with the reagent and sodium hydroxide for 1 hr.

Since the reactivity of the borohydrides increases with the covalent character of the counter ion (6), we investigated the effect of substituting lithium borohydride for the sodium salt in an attempt to find more convenient reaction conditions for 1,4-dien-3-ketosteroids. Experiments with some representative 4-en-3-one, 1,4-dien-3-one, and 4,6-dien-3-one steroids indicated that all of these steroids can be determined by the differential UV method with a reaction time of 10 min. at room temperature, using lithium borohydride in tetrahydrofuran for reduction.

The procedure is as follows: Transfer 1-ml. portions of a well-stirred, 10-mg./ml. suspension of lithium borohydride in tetrahydrofuran to each of two 25-ml. volumetric flasks. Destroy the reducing agent in one flask by adding 5 ml. of methanol and 5 drops of concentrated hydrochloric acid. Transfer 1-ml. portions of a tetrahydrofuran solution of the conjugated 3-ketosteroid under test, containing about 300 mcg./ml., to each flask, mix, and let stand 10 min. with occasional agitation. Destroy excess borohydride in the second flask with 5 ml. of methanol and 5 drops of acid; then dilute both solutions to volume with methanol. Determine the absorbance of the solution from the first flask in a 1-cm. cell at its wavelength of maximum absorbance, using the other solution in the reference cell.

Table I provides a summary of results obtained with this procedure on some authentic samples of steroids. The differential UV spectra of the 4-en-3-one and 1,4-dien-3-one steroids were closely similar to the spectra

Table I—Reduction of Conjugated 3-Ketosteroids with $LiBH_4$ in Tetrahydrofuran

Steroid	Type[a]	Direct UV —in Methanol— $\lambda_{max.}$, nm.	*a*	Differential —Procedure— $\lambda_{max.}$, nm.	*a*
Cortisone	I	241	41.5	241	40.4
Cortisone acetate	I	241	38.6	241	38.0
Hydrocortisone	I	241	42.6	241	41.9
Progesterone	I	241	54.0	241	51.0
Chlormadinone acetate	II	284	49.7	284	49.7
Betamethasone	III	239	40.4	239	37.2
Triamcinolone	III	240	37.0	240	35.9

[a] Type I = 4-en-3-one, II = 4,6-dien-3-one, and III = 1,4-dien-3-one.