Use of 1-Nitroso-2-naphthol in Quantitative Determination of Medicinal Phenolic Compounds

Keyphrases ☐ 1-Nitroso-2-naphthol—determination of medicinal phenolic compounds, UV spectrophotometry ☐ Phenolic compounds, medicinal—determination with 1-nitroso-2-naphthol, UV spectrophotometry ☐ UV spectrophotometry—determination of phenolic compounds after reaction with 1-nitroso-2-naphthol

Sir:

The interaction of 1-nitroso-2-naphthol with tyrosine has long been used to detect the amino acid in biological extracts (1–3). In the presence of a nitric acid solution, this reagent has been successfully applied also to metabolic studies involving the quantitative determination of tyramine (4, 5). However, this reaction has not been applied in pharmaceutical analysis.

The present investigation was prompted by my observation that phenolic compounds like morphine, when treated similarly, gave the characteristic yellow dye, although no maximum absorbance was observed. Under similar conditions, codeine (methylmorphine) did not react with 1-nitroso-2-naphthol. This observation indicated the specificity of the nitrosonaphthol reagent for phenolic compounds.

In the present study, an attempt was made to react various water-soluble, medicinal, phenolic compounds with 1-nitroso-2-naphthol to determine further applications of this reaction. In the future, this information may help us to understand the mechanism of this reaction.

Various phenolic compounds were treated with 1-nitroso-2-naphthol according to the procedure of Udenfriend and Cooper (4). In the general procedure, 0.4 μmole of the phenolic compound is reacted with an excess of 1-nitroso-2-naphthol in the presence of 20% nitric acid at 55° for 30 min. The reaction yields an unstable red derivative which changes to a stable yellow compound. The excess of 1-nitroso-2-naphthol is extracted into ethylene dichloride, and the supernatant aqueous layer is transferred to a 1.0-cm. cell. The visible spectrum of the complex is recorded on a spectrophotometer[1]. The spectra are shown in Fig. 1.

Three different spectra are produced by three different types of phenols, indicating different chromophoric groups of the dye. The 4-hydroxy compounds give $\lambda_{max.}$ at 450 nm., while the 3,5-dihydroxy compounds give $\lambda_{max.}$ at 550 nm. and show a bathochromic shift. However, no increase in molar absorptivity is observed. The 3-hydroxy compounds do produce a dye but fail to give $\lambda_{max.}$ above 350 nm.

Eighteen phenolic compounds were reacted with 1-nitroso-2-naphthol by the procedure described here. These compounds were divided into five classes according to their reactivity (Table I). It is evident from Table I that the compounds of Classes I and II can be quantitatively analyzed. The phenols of Class III cannot be analyzed quantitatively, while the 3,4-dihydroxyphenolic compounds of Class IV do not react with 1-nitroso-2-naphthol. The compounds in Class V do react, but the yellow dye formed seems to be more soluble in ethylene dichloride than in the aqueous phase and, therefore, cannot be analyzed by this procedure.

Table I—Spectral Properties of Dye Produced by Certain Phenolic Compounds

Phenol	Molecular Weight	Molar Absorptivity at 450 nm.
Class I: 4-hydroxyphenolic compounds		
Tyramine hydrochloride	174	4100
Hydroxyamphetamine hydrobromide	232	3828
Methylaminoethanol phenol tartrate	485	2600
Class II: 3,5-dihydroxyphenolic compounds		at 550 nm.
Metaproterenol sulfate	520	3340
Terbutaline sulfate	548	3360
Class III: 3-hydroxyphenolic compounds and metanephrine hydrochloride		
Metanephrine hydrochloride	234	—
Phenylephrine hydrochloride	204	—
Metaraminol bitartrate	336	—
Morphine hydrochloride	322	—
Hydromorphone hydrochloride	323	—
Class IV: unreactive 3,4-dihydroxyphenolic compounds		
Epinephrine hydrochloride	220	—
Norepinephrine hydrochloride	206	—
Nordefrin hydrochloride	220	—
Isoproterenol hydrochloride	248	—
Class V: reactive 4-substituted and 3-substituted phenolic compounds		
Isoxsuprin hydrochloride	328	—
Nylidrin hydrochloride	336	—
p-Cresol	108	—
m-Cresol	108	—

Table II—Assay Results on *p*-Hydroxyamphetamine in Ophthalmic Solution

Experiment Number	Percent of *p*-Hydroxyamphetamine Found in Ophthalmic Solution
1	98.5 100.2
2	98.4 101.3
3	97.8 99.6

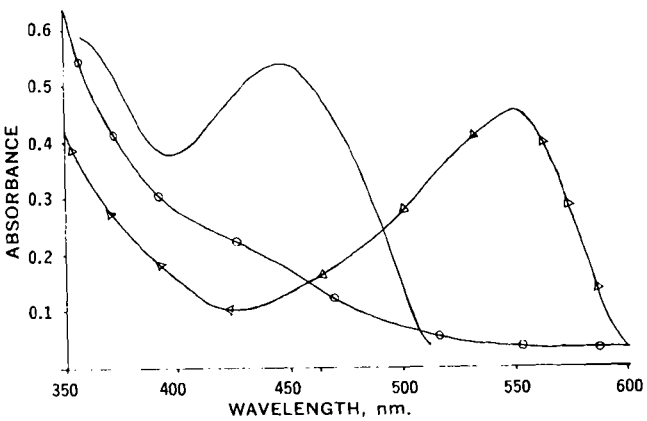

Figure 1—*Visible spectra. Key:* ——, *p-hydroxyphenolic compounds;* Δ-Δ, *3,5-dihydroxyphenolic compounds; and* O-O, *3-hydroxy and similar phenolic compounds.*

[1] Beckman DB-G.

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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(5) K. G. Bhansali, Ph.D. thesis, University of Iowa, Iowa City, Iowa, 1960.

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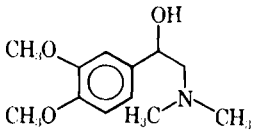
[2] Supplied by Smith Kline & French Laboratories, Philadelphia, Pa.

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



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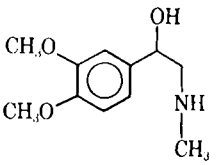

II

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.