Opium Alkaloids XV: Isolation of Stepholidine

E. BROCHMANN-HANSSEN [x] **and W. J. RICHTER** *

Abstract □ A protoberberine alkaloid was isolated from opium and characterized as (−)-2,10-dihydroxy-3,9-dimethoxytetrahydroprotoberberine by spectroscopic methods and by comparison with (−)-stepholidine.

Keyphrases □ Opium alkaloids—isolation and identification of (−)-stepholidine □ (−)-Stepholidine—isolation, identification as opium alkaloid □ Alkaloids, opium—isolation and identification of (−)-stepholidine □ Protoberberine alkaloids—isolation and identification of (−)-stepholidine from opium

In recent years, several new alkaloids have been isolated from opium and the opium poppy or detected by isotope dilution techniques based on established or probable biosynthetic pathways. The phenolic alkaloid fraction has been a particularly rich source of such alkaloids. The present article describes the isolation of a phenolic protoberberine from opium. Alkaloids of this group are found in several plant families. Scoulerine (I) (1) and isocorypalmine (II) (2, 3) were isolated from opium, while coreximine (VI) was detected by an isotope dilution method based on its biosynthesis from reticuline (4).

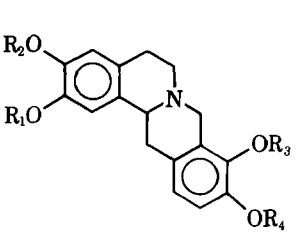
Preparative TLC of the opium fraction containing minor phenolic alkaloids revealed a new alkaloid, which was purified by column chromatography on neutral alumina and crystallized from methanol, mp 135–138°, $[\alpha]_D^{23}$ − 308° (ethanol).

DISCUSSION

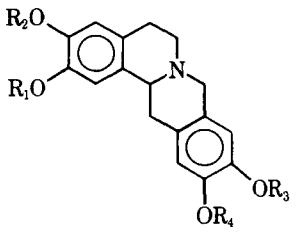
When subjected to GLC analysis[1], the new alkaloid produced a slightly tailing peak with a retention time of 23 min on a 3% OV-1 column at 230°. Silylation[2] gave a bis(trimethylsilyl) derivative (M = 471, determined by mass spectrometry[3]), which formed a sharp, symmetrical peak with increased retention time (31 min) under the same conditions, indicating that two active hydrogen atoms had been present. Correspondingly, the mass spectrum of the alkaloid displayed a molecular ion peak at *m/e* 327, for which high-resolution measurements[4] established a $C_{19}H_{21}NO_4$ composition.

The general fragmentation resembled the pattern characteristic of tetrahydroprotoberberine alkaloids (5–7), with the major fragments at *m/e* 178/177/176 and 150/149, suggesting a dihydroxydimethoxy compound having one hydroxy and one methoxy group in each of the rings A and D. In accordance with this finding, the NMR spectrum[5] in deuterochloroform with internal tetramethylsilane standard also revealed two methoxy singlets resonating at 3.80 and 3.86 ppm and four aromatic protons in the region of 6.5–6.85 ppm.

The optical rotatory dispersion spectrum[6] was very similar to spectra of (−)-scoulerine (I) (8), (−)-isocorypalmine (II)[7], and (−)-tetrahydropalmatine (III) (8). The small amplitude of the second Cotton effect (a = −144 in ethanol) was indicative of a



I: $R_1 = R_3 = H$, $R_2 = R_4 = CH_3$
II: $R_1 = H$, $R_2 = R_3 = R_4 = CH_3$
III: $R_1 = R_2 = R_3 = R_4 = CH_3$
IV: $R_1 = R_4 = H$, $R_2 = R_3 = CH_3$
V: $R_2 = R_4 = H$, $R_1 = R_3 = CH_3$



VI: $R_1 = R_4 = H$, $R_2 = R_3 = CH_3$

2,3,9,10-substitution pattern (8). This finding was confirmed by methylation of the alkaloid with diazomethane, which gave tetrahydropalmatine (III), identified by comparison with authentic material by TLC and GLC. In contrast to scoulerine, the new alkaloid gave no reaction with Gibb's reagent, suggesting that the phenolic functions had no unsubstituted *para*-position.

Mass spectrometric criteria recently developed for assignment of the substitution pattern of ring D of protoberberines, based upon the observation of $(M - OCH_3)^+$ fragments of due abundance[8], finally showed the methoxy group to be located at position 9. For this reason, the compound had to be either stepholidine (IV) (9) or its isomer with reverse substitution in ring A (V)[9]. Identity of the new opium alkaloid with stepholidine was borne out by comparison of the respective IR and mass spectra.

EXPERIMENTAL

Powdered opium of Indian origin [1.8 kg (4 lb)] was extracted, and a preliminary separation of alkaloid groups was carried out as described previously (10). The fraction containing the minor phenolic alkaloid was subjected to preparative TLC on silica gel with chloroform–methanol (9:1) and double development. A broad band, shown by analytical TLC to consist of several alkaloids, was collected, and the alkaloids were eluted from the silica gel with warm methanol. The solution was evaporated to dryness under reduced pressure, and the residue was chromatographed on a column of neutral alumina[10] with chloroform.

Twenty-milliliter fractions were collected with an automatic fraction collector. The first fraction contained porphyroxine, followed by scoulerine, *N*-methyl-14-*O*-desmethylepiporphyroxine, and an unknown alkaloid. The fractions containing this unknown alkaloid were combined and evaporated to dryness under reduced pressure, and the residue (25 mg) was crystallized from methanol, mp 135–138°[11] [lit. (9) 126–138° (*in vacuo*), 158–160° dec.], $[\alpha]_D^{23}$ − 308° (c = 0.192, ethanol) [lit. (9) $[\alpha]_D^{8}$ − 311° in ethanol]; λ_{max} (ethanol): 285 (log ϵ 3.77) and 226 (sh) (4.12) nm. Addition of alkali produced a bathochromic shift.

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(2) S. Pfeifer and J. Teige, *Pharmazie*, **17**, 692(1962).
(3) S. Pfeifer, *ibid.*, **21**, 492(1966).

[1] Varian Aerograph model 2100 gas chromatograph; glass column, 1.4 m (4.5 ft) × 2 mm i.d.
[2] Regisil-TMCS, Regis Chemical Co.
[3] CEC 21-110B mass spectrometer.
[4] The authors thank Dr. A. L. Burlingame and Mr. B. R. Simoneit of the Space Sciences Laboratory, University of California, Berkeley, Calif., for carrying out these measurements.
[5] Varian A-60A NMR spectrometer.
[6] JASCO model ORD/UV-5.
[7] E. Brochmann-Hanssen and K. Hirai, unpublished data.
[8] W. J. Richter and E. Brochmann-Hanssen, to be published.
[9] This isomer was recently shown to be identical with discretamine (W. J. Richter and E. Brochmann-Hanssen, to be published).
[10] Merck, activity 4.
[11] Micro-melting point, Kofler hot stage.

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GLC Determination of Phendimetrazine in Serum

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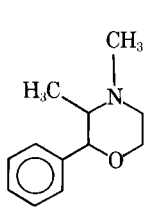
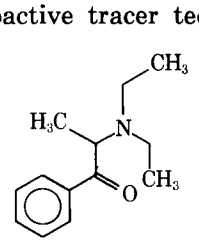
Abstract □ A sensitive, specific, and quantitative GLC method for the determination of phendimetrazine in serum is described. The procedure involves the addition of an internal standard to serum samples, followed by extraction at pH 13 into toluene. The extracted bases are back-extracted into 1 ml of 4 *M* hydrochloric acid and again into 100 μl of chloroform after making the 4 *M* hydrochloric acid extract basic with 1.5 ml of 4 *M* sodium hydroxide. The sensitivity of the method is such that 25 ng of material can be detected in 5 ml of serum

Keyphrases □ Phendimetrazine—GLC analysis in human serum □ GLC—analysis, phendimetrazine in human serum

Phendimetrazine (I) is an anorexigenic agent marketed in South Africa in the form of a standard formulation tablet. To satisfy the requirements of the local Drugs Control Council, the introduction of a timed-release preparation of I on the South African market necessitated the development of a sensitive quantitative method for the detection of I so that serum levels attained after administration of a single timed-release tablet (containing 105 mg of phendimetrazine bitartrate) could be compared with the levels attained after administration of a single tablet of the standard formulation (containing 35 mg of phendimetrazine bitartrate). Although various articles refer to the clinical and toxicological aspects (1–7) of I and its qualitative identification by TLC (8) and GC (9), only one reference dealing with the quantitative determination of phendimetrazine in plasma utilizing a ^{14}C-radioactive tracer technique (10) and one dealing with *in vitro* release studies of timed-release phendimetrazine (11) could be found.



I



II

This report describes a sensitive GLC method for I determinations, under conditions where only I was administered, using diethylpropion (II) as an internal standard.

Table I—Recovery of Phendimetrazine from Serum

Amount Added, ng/ml	Amount Recovered, ng/ml				Mean ± *SD*, ng/ml
20	20.2	20.7	20.9	19.4	20.30 ± 0.67
40	38.9	38.9	37.7	37.9	38.35 ± 0.64
60	59.0	61.2	61.8	62.3	61.08 ± 1.45
80	79.6	80.7	79.5	79.0	79.70 ± 0.71

EXPERIMENTAL

Reagents and Chemicals—The chemicals and reagents used were: 2 *M* sodium hydroxide[1], 4 *M* sodium hydroxide, 4 *M* hydrochloric acid[1], toluene[1], chloroform[1], phendimetrazine bitartrate[2], and diethylpropion hydrochloride[3].

Instrumentation—A gas chromatograph[4] equipped with a flame-ionization detector and a 183-cm × 6-mm glass column containing 3% SE-30 on Chromosorb W (AW HMDS), 80–100 mesh, was used. The injection port and detector were kept at 250°. The column temperature was kept at 140° for 8 min after injection and then programmed at 32°/min to 250°, where it was kept for 8 min. Nitrogen was used as the carrier gas at a flow rate of 80 ml/min. Quantitation was achieved by measuring peak heights. (Peak area calculation by a digital integrator was found to be less reliable.)

Measurement of I in Serum—To 5 ml of serum contained in a 20-ml glass-stoppered centrifuge tube were added 0.5 μg of internal standard (in 50 μl of water), 1 ml of 2 *M* sodium hydroxide, and 5 ml of toluene. The tube was shaken for 2 min (vortex) and centrifuged, and as much as possible of the organic phase was transferred to a 10-ml, glass-stoppered, tapered centrifuge tube contain-

[1] E. Merck, Darmstadt. Pro analysi.
[2] Supplied by Rio Ethicals (Pty.) Ltd.
[3] Supplied by Mer-National Laboratories.
[4] Hewlett-Packard model 5700 A.