TABLE I—ACUTE TOXICITY DATA ON MICE

Dose, mg./Kg.	Mortality
500	0/10
600	1/10
650	5/10
700	6/10
750	8/10

LD_{50} 670 ± 20 mg./Kg.

were determined during a control period and for 6 hr. after the injection of III*a*. The i.v. injection of 2 mg./Kg. and 8 mg./Kg. did not alter the arterial pressure or electrocardiogram or the response to vagal stimulation or challenge injection of the amines. The slight respiratory depression seen was equal to that produced by the suspending agent alone. Similarly, no changes were seen after the i.m. administration of 40 mg./Kg. of III*a*. These results suggest an absence of autonomic and antihistamine effects.

DISCUSSION

The pattern of effects suggests that this compound is not a sedative-hypnotic since it does not produce anesthesia even in large doses. Nor does it have behavioral or autonomic effects suggestive of a tranquilizer of the phenothiazine type. It appears to be similar in its action to thalidomide and trimeglamide to which it is chemically related and to the chemically unrelated 2,5-bis(1,1-dimethyl-3-cyanopropyl)thiazolothiazole (3).

The specific and tragic toxic effect of thalidomide need not obscure the fact that thalidomide defines a purely somnifacient class of drugs potentially safer than the barbiturates. The experience referred to in this paper suggests that drugs of this new class can be recognized during a routine screening and that the action may reside in structures chemically distant from thalidomide.

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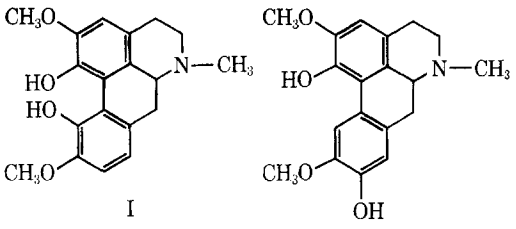
Opium Alkaloids IV. Isolation of Isoboldine

By EINAR BROCHMANN-HANSSEN, BENDIK NIELSEN, and KENTARO HIRAI

The aporphine alkaloid isoboldine has been isolated from opium. It has been identified by NMR and mass spectrometry and by comparison of its infrared spectrum with that of synthetic (±)-isoboldine. ORD measurements have shown that the isolated alkaloid has the absolute configuration corresponding to the (*S*)-series. Possible biosynthetic pathways for isoboldine are discussed briefly.

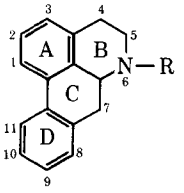
THE APORPHINE alkaloids are widely distributed in nature and, although they occur abundantly in the *Papaveraceae* family, it is only recently that alkaloids of this type have been found in opium. In 1965 Nijland (1) reported the isolation of corytuberine (I) and magnoflorine (*N*-methylcorytuberine). A third member of the aporphine group has now been obtained from opium and identified as isoboldine (II). This alkaloid has previously been isolated from *Nandina domestica* by Tomita *et al.* (2, 3).

All naturally occuring aporphine alkaloids which are known so far are substituted in positions 1 and 2 (III). The amine nitrogen may be secondary, tertiary, or quaternary, and ring D is either unsubstituted or has one or more oxygen functions in positions 8, 9, 10, and 11.



I

II



III

EXPERIMENTAL

Isolation—Dried and powdered opium of Indian origin (1,755 Gm.) was first extracted with petroleum ether, b.p. 40–60°, in a Soxhlet extractor. After drying in air, the opium powder was moistened with an aqueous solution of sodium carbonate (185 Gm. anhydrous Na_2CO_3 in 750 ml. H_2O) and dried

Received October 3, 1966, from the Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco Medical Center, San Francisco, CA 94122

Accepted for publication December 5, 1966.

The authors wish to dedicate this paper to Dean T. C. Daniels, whose leadership at the University of California has done so much to raise the standards of education and research in the pharmaceutical sciences.

This investigation was supported by research grant MH-03487 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

The authors are indebted to Drs. A. H. Jackson and J. A. Martin for a supply of synthetic (±)-isoboldine and to Professor M. Tomita for a sample of natural (+)-isoboldine.

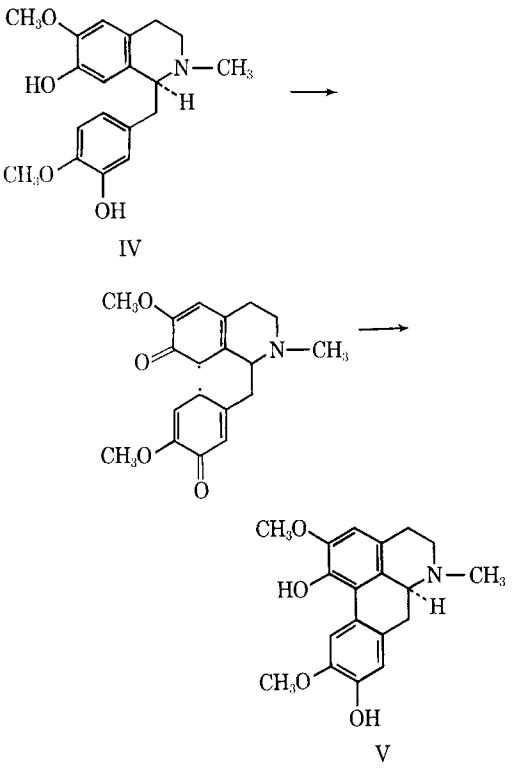
Previous paper: Brochmann-Hanssen, E., and Nielsen, B., *J. Pharm. Sci.*, **55**, 743(1966).

in a vacuum oven at about 30°. The alkalinized material was powdered and extracted with ether in a Soxhlet extractor. The solution in the extraction flask was replaced with fresh ether several times and evaporated to dryness in a rotating vacuum evaporator. The total dry extract obtained in this way weighed 365 Gm. It was dissolved in chloroform and extracted in a separator with McIlvain buffer of pH 6.2. This removed most of the codeine. The chloroform phase was shaken repeatedly with tartaric acid solution (2%), leaving most of the very weak bases (mainly papaverine and narcotine) in the chloroform. When the acid extract was kept in a refrigerator overnight, crystals of thebaine tartrate were formed. These were filtered off and the alkaloids in the filtrate separated into phenolic and nonphenolic bases by extraction with chloroform at pH 13–14. The aqueous layer was adjusted to pH 9–10 with sodium bicarbonate and again extracted with chloroform. After evaporation of the chloroform in a rotating vacuum evaporator, the residue, consisting mainly of diphenolic alkaloids (6.5 Gm.), was subjected to preparative thin-layer chromatography on silica gel (4). Several alkaloid bands could be observed. These were scraped off, the silica gel extracted with warm methanol, and the solutions evaporated under reduced pressure. Two alkaloids obtained in this way were identified as reticuline (2.8 Gm.) and scoulerine (0.5 Gm.) which had previously been isolated from the mother liquors produced during the purification of morphine (5–8). A third alkaloid fraction was crystallized repeatedly from methanol and gave almost colorless crystals (160 mg.) which rapidly became dark when exposed to air, m.p. 125° (micro m.p. K.).

Identification of Isoboldine—The NMR spectrum[1] in deuterochloroform with internal TMS standard revealed three aromatic protons at 2.05, 3.28, and 3.54 τ, two methoxyl groups (singlet, 6.15 τ), an *N*-methyl group at 7.46 τ, and a broad phenolic proton band at 4.3–4.6 τ. Several investigators have studied the nuclear magnetic resonance spectra of aporphine alkaloids and have demonstrated that the NMR spectrum can yield valuable information regarding their substitution pattern (9–11). The low field absorption band (2.05 τ) is characteristic of the aromatic proton at C-11, whereas the bands at 3.28 τ and 3.54 τ are due to the C-8 and C-3 protons, respectively (10). The chemical shift of the methoxyl groups (6.15 τ) indicates that these substituents are located in positions 2, 9, or 10. A methoxyl group at C-1 appears at higher field (6.37–6.58 τ), and a methoxyl group at C-11 has an intermediate chemical shift (6.28–6.35 τ) (9).

When the NMR spectrum of the isolated substance was compared with that of authentic synthetic (±)-isoboldine, the two were found to be identical.

The mass spectrum[2] gave a molecular ion with mass 327. Major fragments appeared in the mass spectrum at *m/e* 326, 312, 296, 284, 269, 253, 165, and 152, corresponding to the favored cleavage of aporphines having an *N*-methyl function (12). The M-31 peak (*m/e* 296) is small, indicating that the alkaloid does not have a methoxyl group in position 1 (12, 13). The M-1 peak (*m/e* 326) is of greater intensity than the peak for the parent ion. This is characteristic of aporphines which are substituted in positions 1,2,9, and 10, while aporphines having a 1,2,10, and 11 substitution pattern exhibit the oppositive relative intensities for these two peaks (14).



Scheme I

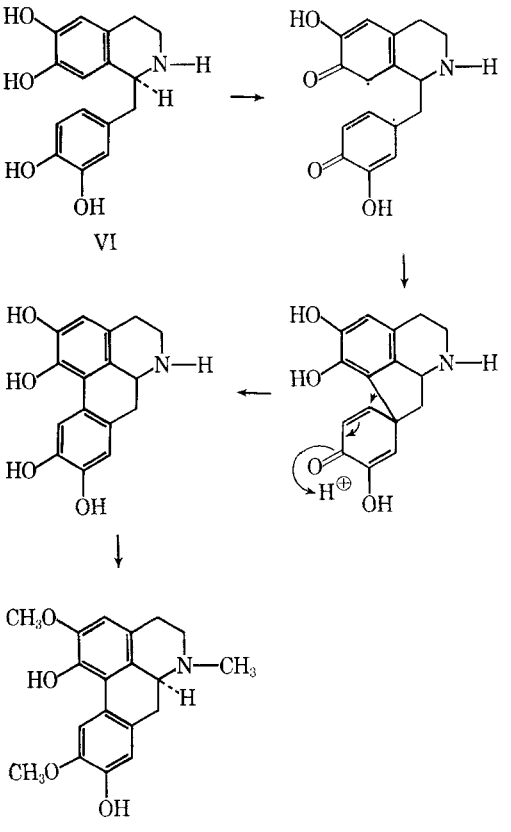
The U.V. spectrum[3] in 95% ethanol gave maxima at 303, 280, and 220 mμ typical of 1,2,9,10 substituted aporphines (15–18). The I.R. spectrum[4] in chloroform was identical with that of authentic (±)-isoboldine.

Stereochemistry—Isoboldine isolated from opium gave a specific rotation of $[\alpha]_D^{25}$ + 66° (c = 0.48 in chloroform). [Lit. + 83.2° (2); + 65.3° (3).] ORD measurements[5] gave a large positive Cotton effect at 251 mμ and a small negative Cotton effect at 320 mμ. From the sign of the Cotton effect in the neighborhood of 250 mμ, it is possible to conclude that the absolute configuration of isoboldine corresponds to the *S*-series according to the Cahn-Ingold-Prelog convention or the L-series if based on the amino acids (19). A sample of isoboldine obtained through the courtesy of Professor Tomita exhibited the same ORD curve as the alkaloid isolated from opium.

DISCUSSION

Robinson (20, 21) was the first to propose that aporphine alkaloids might arise in nature by oxidative coupling of a suitably substituted benzyltetrahydroisoquinoline. Barton and Cohen (22) carried this idea one step further when they suggested that

[1] The instrument used was a Varian HA-100 spectrometer.
[2] Instrument: Associated Electrical Industries, MS9.
[3] Instrument: Unicam SP-800 ultraviolet spectrophotometer.
[4] Unicam SP-200 infrared spectrophotometer.
[5] ORD measurements were made in 95% ethanol at 20° on a Jasco ORD/CD-5 instrument.



VI

Scheme II

the oxidative coupling reaction was the result of a phenol oxidation involving *ortho* or *para* coupling *via* an intermediate quinoid biradical. Two possible mechanisms were envisaged, either a direct coupling to give the aporphine structure, or formation of an intermediate dienone which in turn might rearrange to give rise to a great variety of aporphines (22, 23). The first mechanism is illustrated for isoboldine in Scheme I. The logical precursor is (+)-reticuline (IV) which has the same absolute configuration as (+)-isoboldine (V) (7). It occurs in the opium poppy (7, 24) and has been shown to play an important role in the biosynthesis of a large number of opium alkaloids (25, 26).

An *in vitro* synthesis has been carried out in accordance with the oxidative coupling reaction of Scheme I (27, 28).

If the second mechanism (Scheme II) is used to explain the biosynthesis of isoboldine, a reasonable precursor might be (−)-norlaudanosoline (VI) which also has the correct configuration. Although it has not as yet been isolated from opium or the opium poppy, feeding experiments with radioactively labeled (−)-norlaudanosoline have indicated that it is involved in the biosynthesis of opium alkaloids, presumably as a precursor for (+)-reticuline (25). A closely related mechanism has been demonstrated for the biosynthesis of the aporphine alkaloid isothebaine in *P. orientale* (29).

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