SUMMARY

A rapid and convenient method was developed for the measurement of *p*-methoxycinnamate. With this method, the serum *p*-methoxycinnamate concentration has been measured after oral and intravenous administration to rabbits. *p*-Methoxycinnamate disappeared very rapidly from serum with a half-life of 0.4 hr. when injected intravenously. When orally administered, it was rapidly absorbed into the blood stream, and blood levels peaked within 1 hr. but then declined slowly. Average maximum concentrations were strictly proportional to the oral doses. Its elimination from serum was also first order, but the apparent half-life was more than 2 times that when administered intravenously. At higher doses, however, the elimination was not an exponential process in the initial stage. The metabolite of *p*-methoxycinnamate was identified as *p*-methoxybenzoate by ultraviolet spectrophotometry and paper chromatography.

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Keyphrases

p-Methoxycinnamate
Serum levels of *p*-methoxycinnamate
p-Methoxybenzoate identified as metabolite
UV analysis
Paper chromatographic analysis
Half-life of *p*-methoxycinnamate

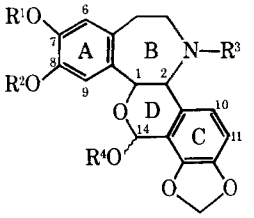
Opium Alkaloids VI

Isolation of *N*-Methyl-14-*O*-desmethylepiporphyroxine

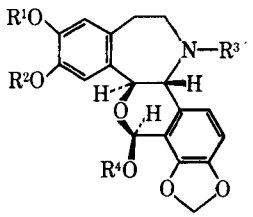
By E. BROCHMANN-HANSSEN, K. HIRAI, B. NIELSEN, S. PFEIFER, I. MANN, and L. KÜHN

The papaverrubine alkaloids and their *N*-methyl derivatives are widely distributed in the genus *Papaver*. They have a number of interesting structural and stereochemical characteristics. A new alkaloid of this general class has now been isolated from opium. It has been characterized by means of NMR and mass spectrometry and by chemical conversions to known compounds.

ALKALOIDS of the general structure (I) are widely distributed in the genus *Papaver* and actually constitute the principal alkaloids of several species (1–3). Many compounds of this type have been isolated from natural sources and differ with regard to their substitution pattern, their conformation, as well as the stereochemistry at the three asymmetric centers. Four opium alkaloids belonging to this group have been reported, namely porphyroxine = papaverrubine D (I; $R^1 = R^4 = CH_3$, $R^2 = R^3 = H$) (4–8), papaverrubine B = *O*-methylporphyroxine (I; $R^1 = R^2 = R^4 = CH_3$, $R^3 = H$) (6, 7, 9, 10), papaverrubine C = epiporphyroxine (I; $R^1 = R^4 = CH_3$, $R^2 = R^3 = H$) (12), and glaudine = *O,N*-dimethylporphyroxine (I; $R^1 = R^2 = R^3 = R^4 = CH_3$) (9, 11). They have a *trans*-



I



II

configuration at the B/D ring junction, whereas other bases of this type, *e.g.*, rhoeadine from

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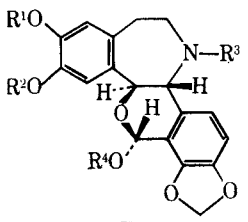
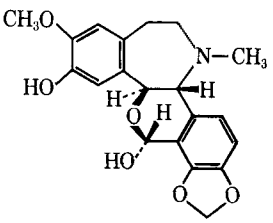
Previous paper: Brochmann-Hanssen, E., and Hirai, K., *J Pharm. Sci.*, **56**, 1658(1967).

P. rhoeas (13) and oreodine from *P. oreophilum* (14, 15) have the C-1 and C-2 protons in *cis*-configuration. There is convincing evidence that the stereochemistry at the 14-position of porphyroxine, papaverrubine B, and glaudine is as shown in II (8). This configuration is thermodynamically unstable and refluxing with methanol containing trace amounts of acid will cause epimerization to III (12, 16). Papaverrubine C already has this configuration (12). However, it may be an artifact formed from porphyroxine during the extraction (12). Treatment with 1 *N* HCl will invert the *trans*-configuration at the B/D ring junction to *cis* (1, 16, 17). Gentle hydrolysis of the methyl acetal glaudine (II; $R^1 = R^2 = R^3 = R^4 = CH_3$) will give the hemiacetal glaucamine (III; $R^1 = R^2 = R^3 = CH_3$, $R^4 = H$) which has been isolated from *P. glaucum* (18). During this hydrolysis, epimerization occurs at the 14-position, and methylation of glaucamine does not produce glaudine but epiglaudine (1, 16) (III; $R^1 = R^2 = R^3 = R^4 = CH_3$).



III



IV

A new phenolic opium alkaloid related to glaucamine has now been isolated simultaneously by research groups at the University of California, San Francisco,[1] and Humboldt University, Berlin, Germany.[2] The present paper is a joint report of the isolation and structure elucidation of this substance. The mass spectrum and accurate mass determinations of the major fragments indicated that the alkaloid was a lactol of structure I in which $R^4 = H$. This was confirmed by NMR spectrometry which showed the presence of one *O*-Me group and a *N*-Me group. The coupling constant $J_{1,2}$ placed the alkaloid as a member of the B/D-*trans*-series. The position of the phenolic hydroxyl group was established by observing the changes in the chemical shifts of the C-6 and C-9 protons in going from the phenol to the phenolate ion. This made it possible to assign structure IV to the new compound. Confirmation of this structure was achieved by methylation of the hydroxyl group in the 14-position to give *N*-methylepiporphyroxine (III; $R^1 = R^3 = R^4 = CH_3$, $R^2 = H$). *O*-Methylation of the new alkaloid with diazomethane produced glaucamine which was further methylated with methanol and acid to epiglaudine. This substance was converted to epiglaudine methiodide with methyl iodide. Glaucamine obtained by methylation of the new alkaloid was refluxed with 1 *N* hydrochloric acid to give the *cis*-isomer (oreogenine) which on methylation produced oreodine. The various derivatives were identified by NMR spectrometry, thin-layer chromatography, melting points, and mixed melting points with authentic materials.

[1] F. Brochmann-Hanssen, K. Hirai, and B. Nielsen.
[2] S. Pfeifer, I. Mann, and L. Kühn.

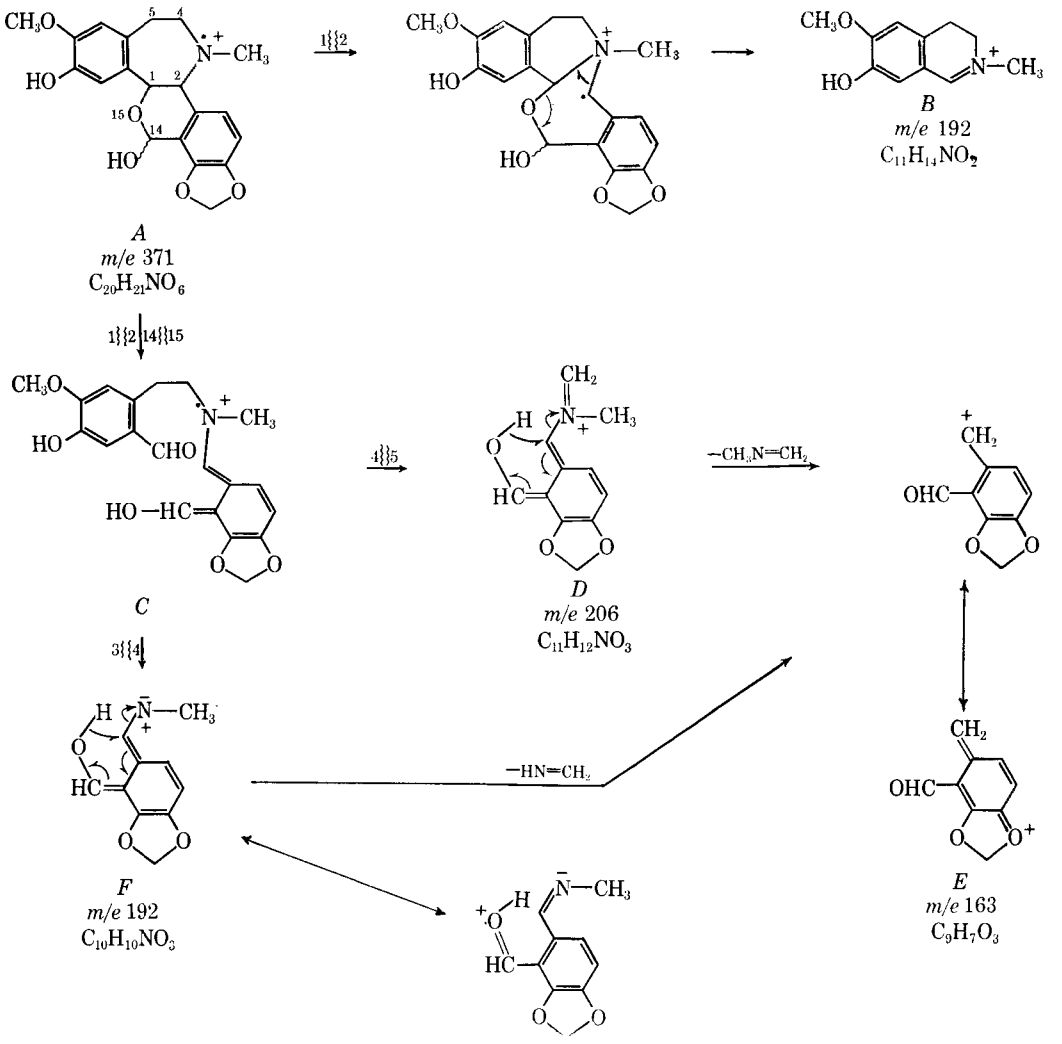
EXPERIMENTAL

Isolation—Dried and powdered opium (1,755 Gm.) of Indian origin was extracted and a separation of the major alkaloid groups carried out by liquid–liquid extraction techniques as described in an earlier publication (19). Preparative thin-layer chromatography of the phenolic alkaloid fraction on silica gel (20) revealed reticuline, isoboldine, scoulerine, and a broad band consisting of several alkaloids. This alkaloid mixture was eluted from the silica gel with warm methanol, the solution evaporated to dryness *in vacuo*, and the residue chromatographed on a column of neutral alumina (Merck, activity 4) with chloroform. Twenty-milliliter fractions were collected with an automatic fraction collector. The first fractions contained porphyroxine (8) followed by scoulerine and several unknown alkaloids. One of these (100 mg.) referred to in the following as A-4 crystallized from methanol as colorless prisms, m.p. 217–218° (micro m.p.K.), $[\alpha]_D^{20} = +340$ (c 0.2% in methanol). It gave a single spot by thin-layer chromatography in two different solvent systems (21) and a single, somewhat broad peak by gas chromatography on silicone rubber SE-30 (3.8%, 4 ft., 200°). When the thin-layer plate was sprayed with potassium iodoplatinate, the orange-brown spot turned to a stable red color after several hours.

Anal.[3]—Calcd. for $C_{20}H_{21}NO_6$: C, 64.73; H, 5.70; N, 3.78. Found: C, 64.32; H, 5.78; N, 4.00.

Elucidation of Structure—The IR spectrum[4] (in Nujol) showed broad bands at about 3270 and 3450 cm.$^{-1}$ due to hydroxyl groups The UV spectrum[5] (in 95% ethanol) had maxima at 238 and 288 mμ which shifted to 242 and 293 mμ on addition of sodium hydroxide, indicating the presence of a phenolic hydroxyl group. The mass spectrum[6] gave a molecular ion with mass 371 and major fragments at *m/e* 206, 192, and 163. The M-1 ion was very small while there was a significant M-18 peak. Metastable ions appeared at *m/e* 138.4 and 129. Accurate mass determinations[7] gave a molecular weight of 371.136908 for the molecular ion

[3] Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley, Calif.
[4] Unicam SP-200 infrared spectrophotometer.
[5] Cary model 11 spectrophotometer.
[6] Associated Electrical Industries, MS 9.
[7] The accurate mass determinations were made by the Robert Robinson Laboratories, University of Liverpool, England.



Scheme I

($C_{20}H_{21}NO_6$ = 371.136879). The *m/e* 206 peak gave a molecular weight of 206.081697 ($C_{11}H_{12}NO_3$ = 206.081712). The *m/e* 192 peak was a doublet: 192.065933 ($C_{10}H_{10}NO_3$ = 192.066063) and 192.101421 ($C_{11}H_{14}NO_2$ = 192.102447) with a 3:1 peak height ratio for the low and the high mass. The *m/e* 163 peak had a mass calculated to 163.039576 ($C_9H_7O_3$ = 163.039515). These results indicated that A-4 was a hemiacetal of the same general structure as glaucamine (22). There is a significant difference in the fragmentation pattern of the acetals (*i.e.*, glaudine and rhoeadine) and hemiacetals (*i.e.*, glaucamine and rhoeagenine). The acetals readily split off $CH_3\cdot$ and $CH_3O\cdot$ to give M-15 and M-31 peaks, whereas the hemiacetals give distinct M-18 ions by a pyrolytic removal of water. The proposed fragmentation of A-4 is illustrated in Scheme I (1, 6, 17, 22). The principal fragmentations occur according to the sequences $A \rightarrow C \rightarrow D \rightarrow E$ and $A \rightarrow C \rightarrow F \rightarrow E$, while the sequence $A \rightarrow B$ represents only a minor pathway. This is consistent with the results obtained by the accurate mass determinations as well as the appearance of metastable ions at *m/e* 138.4 and 129.

The NMR spectra[8] in deuterochloroform and hexadeuterodimethylsulfoxide (internal TMS standard) support the structure IV for A-4. The chemical shifts and the coupling constants are presented in Table I. The C-14 proton of the lactol ring gave a broad signal at 3.71 τ in $CDCl_3$, indicating coupling with an adjacent hydroxyl group. This was confirmed by the addition of D_2O which exchanged with the hydroxyl proton resulting in a sharp peak for the proton in position 14. In $(CD_3)_2SO$ this proton peak overlapped the methylenedioxy signal. The protons in positions 1 and 2 resonated as an AX-type quartet at 6.13 τ (2-H) and 4.26 τ (1-H) in $CDCl_3$ with a coupling constant J = 9 c.p.s. In $(CD_3)_2SO$ this quartet appeared at 6.23 τ and at 4.40 τ, J = 9 c.p.s. These results show that A-4 has a *trans*-configuration at the B/D junction

[8] Varian A-60A NMR spectrometer and C-1024 time averaging computer.

TABLE I—CHEMICAL SHIFTS (τ) AND APPARENT COUPLING CONSTANTS (c.p.s.) OF A-4 AND RELATED COMPOUNDS[a]

Compd.	Solvent	N—CH_3	14-OCH_3	ArOCH_3	14-$OCHO$—[a]	—OCH_2O—	6H	9H	10- and 11-H	2-H	1-H
A-4	$CDCl_3$	7.67	...	6.12	3.71	4.00d, 3.93d $J = 1.2$	3.32	(2.73)[b]	3.27, 3.13, 3.00, 2.87 AB-quartet $J = 8.5$	6.13d $J = 9$	4.26d $J = 9$
	$(CD_3)_2SO$	7.78	...	6.27	3.92	3.97	3.27	2.85	3.11	6.23d $J = 9$	4.40d $J = 9$
Glaucamine (23)	$(CD_3)_2SO$	7.76	...	6.28, 6.25	3.90d $J = 5$	3.98d, 3.90d	3.19	2.85	3.11	6.20d $J = 9$	4.31d $J = 9$
O-Me-A-4	$CDCl_3$	7.70	6.47	6.12	4.30	4.01d, 3.94d $J = \sim 1$	3.33	2.67	3.28, 3.14, 3.02, 2.89 AB-quartet $J = 8.5$	6.01d $J = 9$	4.50d $J = 9$
Epiglaudine (8)	$CDCl_3$	7.70	6.44	6.10 (6H)	4.25	4.02d, 3.95d $J = 1.7$	3.32	2.68	3.28, 3.14, 3.02, 2.88 AB-quartet $J = 8.5$	5.98d $J = 9$	4.43d $J = 9$
N-Me-porphyroxine (8)	$CDCl_3$	7.77	6.32	6.12	4.23	4.05d, 3.93d $J = 1.5$	3.33	2.63	3.27, 3.13, 3.00, 2.86 AB-quartet $J = 8.5$	5.93d $J = 9$	4.83d $J = 9$

[a] d, Doublet. [b] Overlapped with the peak of $CHCl_3$.

TABLE II—CHANGES IN THE CHEMICAL SHIFTS ON ADDITION OF SODIUM DEUTEROXIDE TO A SOLUTION OF A-4 IN $(CD_3)_2SO$ (τ)

	1	2	3	$\Delta\tau$
C-6	3.27	3.34	3.57	0.30 p.p.m.
C-9	2.96	3.06	3.37	0.41 p.p.m.
	phenol → phenolate			

because the coupling constant is in agreement with the values reported for glaucamine (23), isorhoeadine (13, 15), glaudine (15), and porphyroxine (8). A smaller coupling constant ($J_{1,2}$ = 2–2.5 c.p.s.) has been observed for the B/D *cis*-alkaloids (13, 15).

The position of the phenolic hydroxyl group in ring A was determined by measuring the chemical shifts of the aromatic protons as a function of pH as described previously for porphyroxine (8). Small amounts of sodium deuteroxide were added successively to a solution of A-4 in $(CD_3)_2SO$ until no further changes were observed in the chemical shifts of the C-6 and C-9 protons. The results are recorded in Table II. The C-6 proton shifted upfield 0.30 p.p.m. by conversion to the phenolate, and the C-9 proton shifted 0.41 p.p.m. in the same direction.

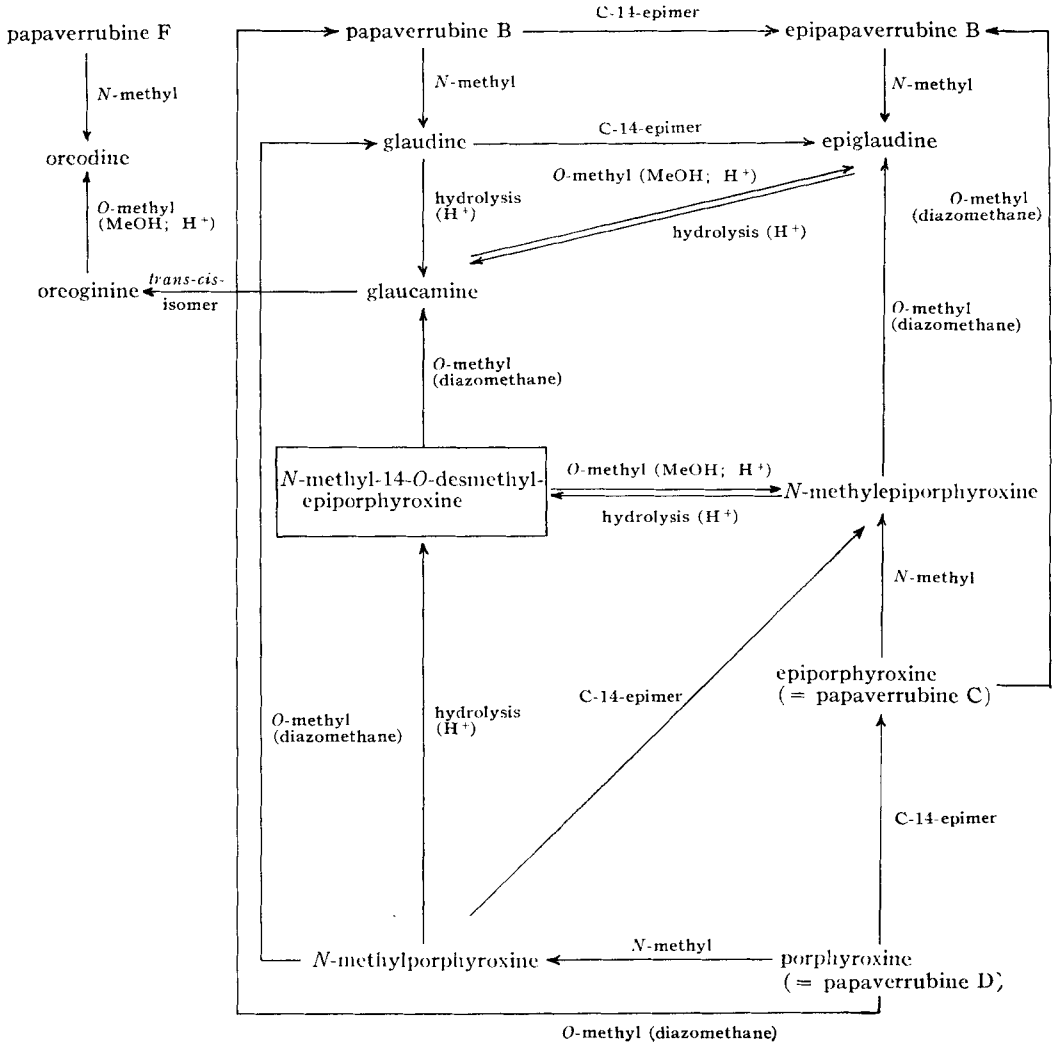
These values are in excellent agreement with those recorded for porphyroxine and show that these two alkaloids have the same substitution pattern in ring A, *i.e.*, the phenolic hydroxyl group is in position 8.

Synthesis of Derivatives

A—*14-O-Methyl-A-4*—Ten milligrams of A-4 was dissolved in 9 ml. of anhydrous methanol and 0.9 Gm. of dry hydrogen chloride was added at 0°. The mixture was allowed to stand at room temperature in the dark for 4 days. The solution was evaporated to dryness *in vacuo*, the residue dissolved in water, made alkaline with sodium bicarbonate, and extracted with chloroform. Evaporation of the chloroform gave an oil which was purified by preparative thin-layer chromatography on silica gel (20). An amorphous substance was obtained which gave a single spot by thin-layer chromatography on silica gel in two solvent systems (21). The R_f values of 14-*O*-methyl-A-4 were identical with those of authentic *N*-methylepiporphyroxine and slightly different from those of *N*-methylporphyroxine. The NMR spectrum of 14-*O*-Me-A-4 was very similar to that of epiglaudine, prepared as described previously (8), but it differed from that of *N*-methylporphyroxine (Table I). It may, therefore, be concluded that 14-*O*-Me-A-4 and *N*-methylporphyroxine only differ with respect to the configuration at the asymmetric center of position-14.

The acetal 14-*O*-methyl-A-4 was also prepared by refluxing for 5 hr. with methanolic hydrochloric acid (0.02 *N*).

B—*Preparation of Glaucamine from A-4*—To a solution of 15 mg. of A-4 in 2 ml. of methanol was added 3 ml. of diazomethane solution (from 0.1 mole of nitrosomethylurea in 100 ml. of ether), and the mixture was kept at 5° for 24 hr. The solution was evaporated to dryness *in vacuo* and the residue dissolved in ether. The solution was concentrated to 1 ml. and allowed to stand for several days to



Scheme II

crystallize. The crystals melted at 222–223° and gave no melting point depression in mixture with authentic glaucamine. The R_f values were identical with those of authentic glaucamine on Silica Gel G (benzene–acetone–methanol, 7:2:1) and on aluminum oxide (Merck) (heptane–chloroform–ether, 4:5:1 and 5:3:2).

C—*Preparation of Epiglaudine Methiodide from A-4*—Glaucamine prepared as described under *B* was refluxed for 5 hr. with methanolic hydrogen chloride (0.02 *N*). The solution was neutralized with ammonia and evaporated to dryness *in vacuo*. The residue was dissolved in a little 0.1 *N* sulfuric acid, immediately made basic with ammonia and extracted with ether. The ether extract was dried over anhydrous sodium sulfate and evaporated to dryness. The residue, which had the same R_f value as authentic epiglaudine, was converted to the methiodide with methyl iodide, m.p. 157–160°. [Lit. m.p. 159–161° (12).]

D—*Preparation of Oreodine from A-4*—Ten milligrams of glaucamine prepared from A-4 as described under *B* was dissolved in 3 ml. of 1 *N* hydrochloric acid and refluxed for 1 hr. After cooling to room temperature, the solution was made basic with ammonia and extracted several times with ether. The combined ether extracts were dried over anhydrous sodium sulfate and evaporated to dryness. The residue (= oreogenine) was dissolved in 5 ml. of methanol containing 0.1 ml. of 1 *N* hydrochloric acid and refluxed for 5 hr. After neutralization with ammonia, the solution was evaporated to dryness *in vacuo*. The residue was dissolved in a little 0.1 *N* sulfuric acid, made basic with ammonia, and extracted repeatedly with ether. The combined ether extracts were dried over anhydrous sodium sulfate and concentrated to 2 ml. The substance, which crystallized as colorless needles, melted at 184–186° and gave no melting point depression when mixed with authentic oreodine. It gave the same R_f values as oreodine by thin-layer chromatography on aluminum oxide (Merck) with two solvent systems prepared from heptane–chloroform–ether (4:5:1 and 5:3:2).

DISCUSSION

A new phenolic alkaloid has been isolated from opium and characterized as *N*-methyl-14-*O*-desmethylepiporphyroxine (IV) by mass and NMR spectroscopy and by chemical conversion to known compounds. It belongs to a group of plant bases of the general structure I which is widely distributed in the genus *Papaver*. *N*-Methyl-14-*O*-desmethylepiporphyroxine is the fifth such alkaloid isolated from opium. Like glaucamine, it is a lactol (hemiacetal) having a thermodynamically stable configuration at the 14-position, and a *trans*-configuration at the B/D ring junctions. The relationships of the new base to other compounds of this type have been verified experimentally and are illustrated in Scheme II. *N*-Methyl-14-*O*-desmethylepiporphyroxine may also be obtained by hydrolysis of *N*-methylporphyroxine. There is reason to believe that *N*-methylporphyroxine, having the less stable configuration at the 14-position (II) may occur in the opium poppy. It is, therefore, possible that *N*-methyl-14-*O*-desmethylepiporphyroxine may not exist as such in the fresh plant, but may be formed by acid hydrolysis and epimerization during the drying and storage of opium or in the course of the extraction and purification of the alkaloid fractions.

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Keyphrases

Opium alkaloids
N-Methyl-14-*O*-desmethylepiporphyroxine identification
TLC separation
Potassium iodoplatinate reagent
NMR spectrometry
Mass spectrometry
UV spectrophotometry—structure
IR spectrophotometry—structure
Synthesis of *N*-methyl-14-*O*-desmethylepiporphyroxine derivatives