# Structure and Configuration of Alkaloids. II. Cassine[1,2]

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Cassine has been shown to be 2-(*R*)-methyl-3-(*R*)-hydroxy-6-(*S*)-(11-oxododecyl)piperidine by the preparation of the mirror image of N-methylcassine from the alkaloid carpaine which is of known absolute configuration.

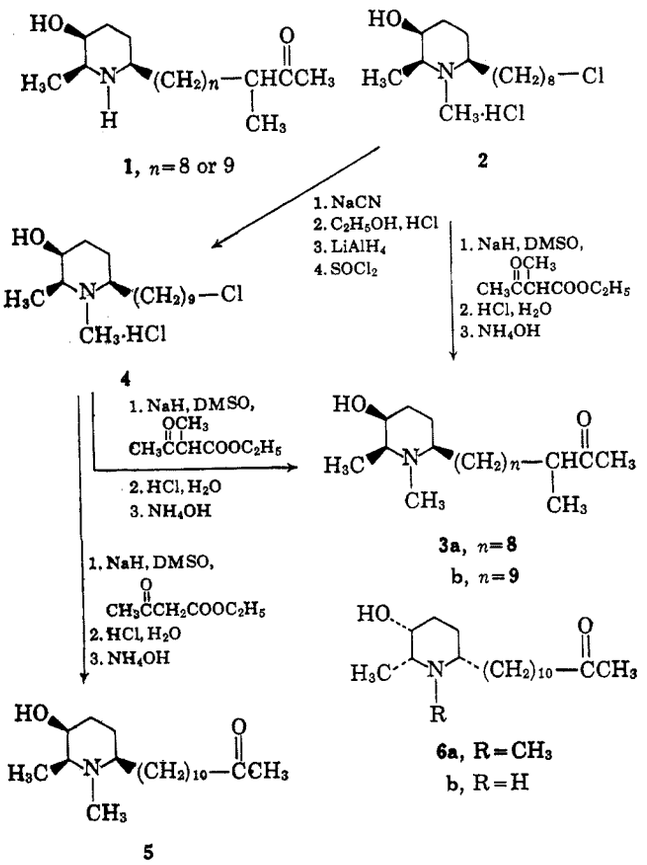
Recently a paper was published[5] on the alkaloids present in *Cassia excelsa* Shrad. One of these alkaloids, cassine, was investigated in some detail. In order to fit the experimental results into some sort of logical structure for future work, Highet proposed the tentative structure **1** (or mirror image) for cassine. Evidence for the ring structure and stereochemistry was quite good, but evidence for the structure of the side chain was of a negative type (exchange of only 3.7 deuteriums for hydrogen and lack of formation of a dibenzylidine derivative). This left some doubt as to the exact structure of the side chain in cassine.

In our work on the absolute configuration of the alkaloid carpaine we prepared compound **2**, the absolute configuration of which is now known.[1] We decided to use compound **2** to prepare compounds corresponding to the various structures that were proposed for N-methylcassine (**3a** or **3b** or the mirror image of these).[5]

In an attempt to synthesize N-methylcassine from carpaine, we used compound **2** to alkylate ethyl $\alpha$-methylacetoacetate. The product of this reaction was hydrolyzed and decarboxylated with aqueous hydrochloric acid to give compound **3a** (Chart I). In order to extend the side chain on compound **2**, to prepare the other possible structure suggested for N-methylcassine, we allowed compound **2** to react successively with sodium cyanide, ethanolic hydrogen chloride, lithium aluminum hydride, and finally with thionyl chloride to obtain compound **4**. Ethyl $\alpha$-methylacetoacetate was then alkylated with compound **4** and the product was treated with aqueous hydrochloric acid to get compound **3b**. Compounds **3a** and **3b** can each exist as two possible epimers and we do not know whether or not we have isolated a mixture in each case. The hydrochlorides of compounds **3a** and **3b** have melting points which correspond reasonably well to that of N-methylcassine hydrochloride, but the optical rotations do not correspond. The infrared spectra of the hydrochlorides of **3a** and **3b** are similar to that of N-methylcassine hydrochloride, but there are distinct differences in each case.

The interesting thing about compounds **3a** and **3b** is their nmr spectra. In addition to the N-methyl group at $\tau$ 7.90, these compounds show a three-peak multiplet at high field (centered at about $\tau$ 8.93) where cassine shows only a doublet centered at about $\tau$ 8.98. We attribute these three peaks to two overlapping doublets due to the C-methyl on the $\alpha$-position of the ketone and the ring C-methyl. This led us to suspect that cassine

CHART I




probably has a straight side chain with no branching. Highet very kindly confirmed this suspicion by informing us of his recent work,[6] which proves that cassine has a straight 12-carbon side chain. Thus N-methylcassine is more likely compound **5** or its mirror image. Compound **5** was prepared by alkylation of ethyl acetoacetate with compound **4** followed by hydrolysis and decarboxylation of the product.

Compound **5** shows an nmr spectrum that is almost identical with that of cassine except for the N-methyl group at $\tau$ 7.90 replacing the hydrogen on the nitrogen of cassine. The doublet corresponding to the C-methyl on the piperidine ring has been shifted downfield so that it is centered at about $\tau$ 8.87 in compound **5**, whereas it was centered at $\tau$ 8.98 in cassine.

There are two crystalline derivatives of compound **5** that are important for comparison with those of N-methylcassine. These are the hydrochloride, $[\alpha]^{23}D$ $-6.7°$, and the methiodide, $[\alpha]^{23}D$ $-16.3°$. The rotations of these compounds are almost identical in magnitude with those of the same derivatives of N-methyl-

(1) Paper I: J. L. Coke and W. Y. Rice, Jr., *J. Org. Chem.*, **30**, 3420 (1965).

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(5) R. J. Highet, *J. Org. Chem.*, **29**, 471 (1964).

(6) R. J. Highet and P. F. Highet, *ibid.*, **31**, 1275 (1966). We wish to thank these authors for informing us of their results prior to publication.

cassine[5] (hydrochloride, $[\alpha]^{26}D$ 6.5°, and methiodide, $[\alpha]^{26}D$ 15.8°) but are of opposite sign. The infrared spectra of the hydrochloride of **5** and N-methylcassine hydrochloride are superimposable. The infrared spectra of the methiodide of **5** and N-methylcassine methiodide are superimposable, and the two compounds show identical behavior and retention times on gas chromatography.[7] This indicates that compound **5** is the mirror image of N-methylcassine. Thus N-methylcassine must have structure **6a** and cassine must have structure **6b**.

## Experimental Section[8]

**N-Methyl-2-(*S*)-methyl-3-(*S*)-hydroxy-6-(*R*)-(8-chlorooctyl)-piperidine Hydrochloride** (2).—An excess of dry hydrogen chloride was passed into an ether solution of 6.46 g (0.025 mole) of N-methylcarpamdiol[1] until no more material separated, and the ether was then removed under vacuum. The resulting hydrochloride was dissolved in 40 ml of ethanol-free chloroform. The solution was cooled in an ice bath while 2.0 ml (0.028 mole) of thionyl chloride was added. The reaction mixture was stirred for 74 hr at room temperature and was worked up by addition of 100 ml of 10% sodium carbonate solution and several extractions with chloroform. The chloroform solutions were combined, dried, and evaporated under vacuum to give 7.5 g of an oil, which was chromatographed over 200 g of neutral grade I Woelm alumina. Elution with 1250 ml of chloroform gave 6.0 g of an oil which was pure by thin layer chromatography.[9] This oil was dissolved in ether and treated with dry hydrogen chloride to give a solid which was collected by filtration and crystallized from ethanol–ether to give 5.77 g (74% of theory) of product, mp 128–130°. An analytical sample was prepared by recrystallization from ethanol–ether, mp 130–132°, $[\alpha]^{23}D$ −4.5° (*c* 0.98 g/100 ml, absolute ethanol).

*Anal.* Calcd for $C_{15}H_{31}Cl_2NO$: C, 57.68; H, 10.01; Cl, 22.70; N, 4.49. Found: C, 57.83; H, 10.22; Cl, 22.65; N, 4.61.

**N-Methyl-2-(*S*)-methyl-3-(*S*)-hydroxy-6-(*R*)-(8-cyanooctyl)-piperidine Hydrochloride.**—A solution of 2.79 g (0.0101 mole) of compound **2** and 1.10 g (0.0225 mole) of sodium cyanide in 10 ml of dry dimethyl sulfoxide was stirred at 135° under nitrogen for 1.5 hr. The resulting solution was cooled and diluted with 150 ml of water and then extracted with three 25-ml portions of ether. The ether solutions were combined, washed with saturated sodium chloride solution, dried, and evaporated to give 2.66 g of oil. This oil showed only one spot on thin layer chromatography.[9] The oil was dissolved in ether and treated with dry hydrogen chloride to give a hydrochloride, which was crystallized from ethanol–ether to give 2.60 g (85% of theory) of product, mp 138–140°.

*Anal.* Calcd for $C_{16}H_{31}ClN_2O$: C, 63.44; H, 10.32; N, 9.25. Found: C, 63.44; H, 10.33; N, 9.41.

**N-Methyl-2-(*S*)-methyl-3-(*S*)-hydroxy-6-(*R*)-(9-chlorononyl)-piperidine Hydrochloride** (4).—A solution of 2.60 g (0.0086 mole) of the nitrile hydrochloride from the preceding experiment in 25 ml of 95% ethanol and 5 ml of concentrated hydrochloric acid was heated at reflux for 13 hr. The solvent was removed under vacuum. The resulting oil was dissolved in 50 ml of absolute ethanol and the solution was saturated with dry hydrogen chloride and heated at reflux for 58 hr. The solvent was removed under vacuum and the residue was basified with cold 10% potassium carbonate. Extraction with ether gave a solution which was dried and evaporated to give 2.38 g of crude ester, which showed no absorption at 2245 $cm^{-1}$ in the infrared but did show strong absorption at 1735 $cm^{-1}$. This ester was not purified further but was reduced directly to diol as follows. A solution of 3.0 g (0.0096 mole) of ester in 100 ml of ether was added to 0.50 g (0.013 mole) of lithium aluminum hydride, and the mixture was stirred at room temperature for 44 hr. The reaction mixture was worked up by successive slow addition of 0.5 ml of water, 0.4 ml of 20% sodium hydroxide solution, and 1.8 ml of water. The ether was decanted from the solid aluminum hydroxide and the solids were washed well with ether. All the ether solutions were combined, dried, and evaporated to give 2.68 g of oil, which showed no absorption at 1735 $cm^{-1}$ in the infrared but did show strong hydroxyl absorption. This oil was chromatographed over 100 g of neutral grade I Woelm alumina. Elution with 560 ml of chloroform followed by 475 ml of chloroform containing 2% methanol gave a total of 2.0 g (77% of theory) of pure diol (one spot on thin layer chromatography[9]), which was not further purified but was converted directly to compound **4** as follows. The diol (2.0 g, 0.0074 mole) was converted to the hydrochloride and subsequently to the monochloro hydrochloride **4** in a manner identical with that used in the preparation of compound **2**, except that 0.62 ml (10% excess) of thionyl chloride was used. Pure compound **4** (0.76 g) was obtained by crystallization of the product from ethanol–ether: mp 138–140°, $[\alpha]^{23}D$ −5.8° (*c* 1.15 g/100 ml, absolute ethanol).

*Anal.* Calcd for $C_{16}H_{33}Cl_2NO$: C, 58.88; H, 10.19; Cl, 21.37; N, 4.29. Found: C, 58.90; H, 10.02; Cl, 21.59; N, 4.43.

**Alkylation Procedures.**—The following procedure is representative of the general alkylation procedures used on the chloro compounds **2** and **4** so it is given in detail only once.

**A. N-Methyl-2-(*S*)-methyl-3-(*S*)-hydroxy-6-(*R*)-(11-oxododecyl)piperidine** (5).—A solution of 0.702 g (2.15 mmoles) of **4** in 10 ml of dimethyl sulfoxide was added dropwise to a stirred solution of 2.04 g (15.4 mmoles) of ethyl acetoacetate and 0.202 g (8.4 mmoles) of sodium hydride in 10 ml of dimethyl sulfoxide maintained at 130°. The resulting solution was stirred at 130° for 2.2 hr and was then cooled and diluted with 200 ml of water. The aqueous mixture was extracted repeatedly with ether. The ether solutions were combined, washed with saturated sodium chloride solution, and were then extracted with two 20-ml portions of 10% hydrochloric acid. The acid extracts were combined and carefully basified with concentrated aqueous ammonia with cooling. The basic mixture was extracted well with chloroform and the combined extracts were dried and evaporated under vacuum to give 0.8 g of viscous oil. This oil was hydrolyzed and decarboxylated by dissolving it with 10 ml of concentrated hydrochloric acid and letting the solution stand at room temperature for 72 hr, then diluting it with 10 ml of water and heating it on a steam bath for 2 hr. After washing the acidic solution with ether to get rid of nonbasic compounds, the solution was basified with concentrated aqueous ammonia and extracted several times with chloroform. The chloroform solutions were combined, dried, and evaporated to give 0.52 g of oil which was chromatographed over 50 g of neutral grade I Woelm alumina. Elution with 200 ml of chloroform gave 0.28 g of pure **5** as an oil which showed strong absorption at 3450 and 1720 $cm^{-1}$ in the infrared spectrum. The nmr spectrum of compound **5** showed the following peaks: $\tau$ 7.90 (N-methyl), 7.97 (methyl ketone), 8.70 (broad, methylene), and doublet centered at $\tau$ 8.87 (ring C-methyl).

A 200-mg sample of compound **5** in ether was converted to the hydrochloride with dry hydrogen chloride. This hydrochloride was crystallized from ethyl acetate: mp 103–105.5°, $[\alpha]^{23}D$ −6.7° (*c* 1.49 g/100 ml, absolute ethanol).

*Anal.*[10] Calcd for $C_{19}H_{38}ClNO_2$: C, 65.58; H, 11.01; N, 4.03. Found: C, 64.72; H, 11.03; N, 3.88.

The reported[5] constants for N-methylcassine hydrochloride are mp 110.5–111.5°, $[\alpha]^{26}D$ 6.5°. The infrared spectra of the

(7) We wish to thank Fr. R. J. Highet for comparing the spectra of the methiodides and for doing the gas chromatographic comparisons. Dr. Highet suggests that the methiodides probably demethylate under the conditions used for gas chromatography.

(8) All melting points were taken on a calibrated Kofler hot stage. Nmr spectra were taken in carbon tetrachloride on a Varian A-60 spectrometer with TMS as an internal standard. Infrared spectra were taken on a Perkin-Elmer Model 237 B grating Infracord using KBr disks for solids and neat films for liquids. Optical rotations were taken with a Rudolph photoelectric polarimeter, Model 200.

(9) All purity checks on compounds using thin layer chromatography were run using alumina as the adsorbent and the following eluents: chloroform, 2% methanol in chloroform, benzene, and 2% methanol in benzene.

(10) No satisfactory carbon analysis could be obtained for the hydrochlorides of compounds **3a**, **3b**, or **5** even on repeated analyses using highly purified material. The carbon analyses were always low. Dr. Highet has noted the same analytical difficulty. This problem seems to be peculiar to the ketones, because precursors of these compounds gave good analyses for carbon.

hydrochloride of compound **5** and N-methylcassine hydrochloride are superimposable.[11]

A 200-mg sample of compound **5** was converted to the methiodide using 3 ml of absolute ethanol and 2 ml of methyl iodide. The reaction was allowed to proceed at room temperature overnight and the volatile material was removed under vacuum. The residue was crystallized twice from ethyl acetate to give the very hygroscopic methiodide, mp 79–84°, $[\alpha]^{23}D$ −16.3° (*c* 1.49 g/100 ml, absolute ethanol).[11] No analysis was attempted because of the hygroscopic nature of this compound.[7]

The reported physical constants for N-methylcassine methiodide are[5] mp 91–93°, $[\alpha]^{26}D$ 15.8°.

**B. N-Methyl-2-(*S*)-methyl-3-(*S*)-hydroxy-6-(*R*)-(10-oxo-9-methylundecyl)piperidine (3a).**—The same alkylation procedure was employed here as in part A except that 1.81 g (12.6 mmoles) of ethyl α-methylacetoacetate, 0.322 g (13.3 mmoles) of sodium hydride, and 1.54 g (4.95 mmoles) of N-methyl-2-(*S*)-methyl-3-(*S*)-hydroxy-6-(*R*)-(8-chlorooctyl)piperidine hydrochloride (**2**) were used. The yield of product was 0.45 g. The nmr spectrum of compound **3a** was almost identical with that of compound **5** except that **3a** showed a three-peak multiplet centered at about τ 8.93 (the center peak was very slightly resolved), where **5** showed a doublet centered at τ 8.87.

Compound **3a** gave a crystalline hydrochloride which was crystallized from ethyl acetate, mp 103–106°, $[\alpha]^{24}D$ −8.9° (*c* 1.07 g/100 ml, absolute ethanol). The infrared spectrum of this compound was not identical with that of N-methylcassine hydrochloride.

*Anal.* Calcd for $C_{19}H_{38}ClNO_2$: C, 65.58; H, 11.01; Cl, 10.19; N, 4.03. Found: C, 64.96; H, 11.04; Cl, 10.01; N, 4.26.

**C. N-Methyl-2-(*S*)-methyl-3-(*S*)-hydroxy-6-(*R*)-(11-oxo-10-methyldodecyl)piperidine (3b).**—The same alkylation procedure was employed here as in part A except that 2.00 g (13.9 mmoles) of ethyl α-methylacetoacetate, 0.200 g (8.33 mmoles) of sodium hydride, and 0.576 g (1.77 mmoles) of N-methyl-2-(*S*)-methyl-3-(*S*)-hydroxy-6-(*R*)-(9-chlorononyl)piperidine hydrochloride (**4**) were used. The yield of product was 0.25 g. The nmr spectrum of this compound was almost identical with that of compound **3a**.

Compound **3b** gave a crystalline hydrochloride which was crystallized from methanol–ether, mp 105–114°, $[\alpha]^{24}D$ −10.4° (*c* 1.03 g/100 ml, absolute ethanol). The infrared spectrum of this compound was not identical with that of N-methylcassine hydrochloride.

*Anal.* Calcd for $C_{20}H_{40}ClNO$: C, 66.36; H, 11.06; N, 3.88. Found: C, 65.88; H, 11.35; N, 4.19.

**Acknowledgment.**—We wish to thank Drs. R. K. Hill and R. J. Highet for helpful discussions concerning this work. We are especially grateful to Dr. Highet for supplying us with a sample of N-methylcassine hydrochloride and for his help in comparing physical constants and spectra of our compounds.

(11) We should comment on the observed melting points of our derivatives compared with those reported by Highet.[5] The hydrochloride of compound **5** has a melting point of 103–105.5°. This is below that reported in the literature for N-methylcassine hydrochloride (lit. mp 110.5–111.5°) but an authentic sample of N-methylcassine hydrochloride supplied by Dr. Highet showed a melting point of 104–106.5° on our apparatus. The methiodide of compound **5** has a melting point of 79–84°, whereas N-methylcassine methiodide is reported to have a melting point of 91–93°. We have been unable to purify the methiodide of **5** sufficiently or keep it dry enough to obtain a good melting point. The compound is extremely hygroscopic. We feel that the methiodide of **5** is pure enough to have a fairly reliable specific rotation but not to have a very good melting point, primarily because of its hygroscopic nature.

# The Mitomycin Antibiotics. Synthetic Studies. XIII.[1a] Indoloquinone Analogs with Variations at C-5

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2,6-Dimethyl-1-ethyl-4-hydroxyindole (IIIa) was converted into the corresponding 3-formyl derivative VIIIa *via* acetylation, formylation, and deacetylation. The latter compound reacted poorly with Fremy's salt, but by employing an excess of this reagent at mildly elevated temperatures it could be oxidized in satisfactory yield to the corresponding 4,7-quinone (XIa). Under these conditions an oximino derivative (XII) of XIa was also isolated, presumably as a result of breakdown products of Fremy's salt combining with XIa. An alternate synthesis of XIa involving Fremy's salt oxidation of IIIa prior to formylation proved infeasible. Conversion of XIa to 3-hydroxymethyl methylcarbamate Xa was effected by standard procedures. By a route parallel to that just described the corresponding 5-methyl homolog Xb was also prepared. The antibacterial and antifungal activities of the above two carbamates are noted.

As part of our comprehensive program for preparing analogs of active indoloquinones (*e.g.*, I)[2] related to the mitomycin antibiotics, structures with variation of the substituent at C-5 of the quinone ring were important. In this paper we describe the preparation of analogs wherein the 5-methoxy group of I has been replaced by hydrogen and methyl.

For the 5-hydrogen analog Xa two routes parallel to those employed for the corresponding 5,6-unsubstituted analog II[1a] were envisioned, starting from 2,6-dimethyl-1-ethyl-4-hydroxyindole (IIIa).[3] However, the route preferred for the preparation of II was shown to be inapplicable to the present objective when it was not possible to effect in acceptable yield the first transformation in the sequence, namely, oxidation to *p*-quinone V. Thus, Fremy's salt (potassium nitrosodisulfonate)[4] treatment of IIIa afforded only 5% of V and 5% of *o*-quinone VII. In contrast, yields of 68 and 12% had been obtained for the corresponding *p*- and *o*-quinones, respectively, in the 5,6-unsubstituted series.[1a] In view of this difficulty we directed our efforts toward developing the alternate pathway. Hydroxyindole IIIa was converted to its acetate IVa and the latter compound was formylated under Vilsmeier–Haack conditions.[5] Deacetylation of the resulting 3-formyl-4-acetate VIa afforded 3-formyl-4-hydroxyindole VIIIa. Fremy's salt oxidation of VIIIa gave a result similar to that obtained with the corresponding 3-formyl-5,6-unsubstituted compound[1] in that the conversion of starting material to *p*-quinone XIa

(1) (a) Preceding paper in this series: W. A. Remers and M. J. Weiss, *J. Am. Chem. Soc.*, **88**, 804 (1966); (b) to whom inquiries should be directed.

(2) G. R. Allen, Jr., J. F. Poletto, and M. J. Weiss, *J. Am. Chem. Soc.*, **86**, 3878 (1964).

(3) W. A. Remers and M. J. Weiss, *ibid.*, **87**, 5262 (1965).

(4) See H. J. Teuber and G. Jellinek, *Ber.*, **85**, 95 (1952), and subsequent papers.

(5) A. Vilsmeier and A. Haack, *ibid.*, **60**, 119 (1927).