Acknowledgment. This investigation was supported by Grant 5-RO1-CA15824, awarded by the National Cancer Institute, Department of Health, Education, and Welfare.

Registry No.—**2a,** 601-95-6; **2b,** 63533-72-2; **2c,** 63533-73-3; **2d,** 61543-88-2; **2e,** 63533-74-4; **2f,** 63533-75-5; **3c** isomer 1, 63533-76-6; **3c** isomer 2, 63533-77-7; **4a,** 61543-93-9; **4b,** 63533-78-8; **4c,** 63533-79-9; **4d,** 63533-80-2; **4e,** 63533-81-3; **4f,** 63533-82-4; **4g,** 63533-83-5; **5a,** 63533-84-6; **5b,** 63533-85-7; **5c,** 63533-86-8; **6,** 63533-87-9; **7a,** 63533-88-0; **7b,** 63533-89-1; **7c,** 62251-60-9; **7d,** 63547-45-5; **7e,** 63533-90-4; **7f,** 63533-91-5; **8a,** 63533-92-6; **8c,** 63533-93-7; **8d,** 63533-94-8; **9a,** 63533-95-9; **9b,** 63533-96-0; **9c,** 63533-97-1; **10b,** 63533-98-2; **10c,** 63533-99-3.

References and Notes

(1) For Parts III and IV, refer to J. R. Dias and R. Ramachandra, *Synth. Commun.*, **7**, 293 (1977), and *Org. Prep. Proc. Int.*, **9**, in press.
(2) Postdoctoral Research Associate, 1974–1977.
(3) J. R. Dias and R. Ramachandra, *J. Org. Chem.*, **42**, 1613 (1977).
(4) J. Baker and R. T. Blickenstaff, *J. Org. Chem.*, **40**, 1579 (1975).
(5) Also, we discovered that nitrate esters, like 3β-nitroxy-5α-cholestane, undergo quantitative HNO_3 elimination in hot HMPT which is currently under investigation in our laboratory.
(6) L. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **72**, 5530 (1950).
(7) I. Tanasescu, F. Hodosan, and I. Jude, *Chem. Ber.*, **91**, 799 (1958).

Protoberberine Alkaloids. Structures of Aequaline, Coramine, Discretinine, and Schefferine

Einar Brochmann-Hanssen* and Hsüch-Ching Chiang

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California, 94143, and School of Pharmacy, National Taiwan University, Taipei, Taiwan, Republic of China.

Received April 11, 1977

The structures assigned to the protoberberine alkaloids aequaline and coramine were found to be incorrect. Instead, aequaline was shown to be identical with discretamine (**6**), and coramine was identical with coreximine (**13**). Schefferine was found to have the same structure as kikemanine [(−)-corydalmine] (**8**), and discretinine was shown to be corypalmine (**14**) by comparison with authentic samples.

The protoberberine alkaloids are widely distributed in many plant families, mainly as the tetrahydroprotoberberines and the quaternary protoberberine salts.[1-4] They are biosynthesized from benzyltetrahydroisoquinolines[5-7] and, in turn, serve as biosynthetic intermediates for many other alkaloid groups.

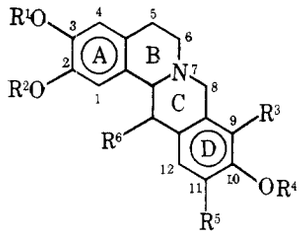
The assignment of the substitution pattern of protoberberines isolated from natural sources has often presented considerable problems, especially when insufficient material has been available for chemical degradations. Spectroscopic data can give valuable information,[8] but the final proof of structure comes from chemical synthesis. Several protoberberine alkaloids have been isolated whose structures are still not known in all detail, and there are others which have been assigned incorrect structures.

In 1972 two tetrahydroprotoberberine alkaloids were isolated from the bark of *Schefferomitra subaequalis* and named aequaline and schefferine.[9] Both alkaloids were levorotatory and gave (−)-tetrahydropalmatine (**1**) on methylation with diazomethane, thereby establishing a 2,3,9,10-tetraoxygenated substitution pattern. Elemental analysis of aequaline gave the molecular formula $C_{19}H_{21}NO_4$. The NMR spectrum established the presence of two methoxyl and two hydroxyl groups, and mass spectroscopy showed that both rings A and D each had one hydroxyl and one methoxyl group. A 9-hydroxy-10-methoxy substitution was suggested based on the relative abundances of the fragments. Since aequaline was shown by direct comparison to be different from scoulerine (**2**), the structure of aequaline was proposed to be (−)-3,9-dihydroxy-2,10-dimethoxytetrahydroprotoberberine (**3**).

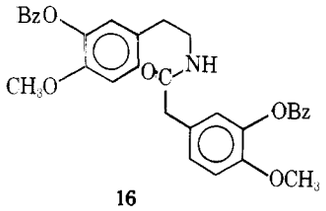
Microanalysis of the second alkaloid, schefferine, gave a molecular formula $C_{20}H_{23}NO_4$ and a molecular ion peak *m/e* 341 in its mass spectrum indicating the presence of one hydroxyl and three methoxyl groups. Based on the fragmentation pattern, two methoxyl groups could be assigned to ring A. Since monomethylation of aequaline with diazomethane gave schefferine as one of the products, structure **4** was assigned to schefferine.

Recently, mass spectrometric criteria were developed for detecting a methoxyl group in position 9 of protoberberine alkaloids based on the abundance of the $(M - OCH_3)^+$ fragment compared to that of the molecular ion.[8] Compounds with a 9-methoxy substituent give a $(M - OCH_3)^+$ fragment ranging from 12 to 19% of the molecular ion. If the compounds are either unsubstituted in position 9 or have a 9-hydroxy substituent, the relative abundance of the $(M - OCH_3)^+$ fragment is <3% of the molecular ion peak. Preliminary mass spectroscopic studies[10] have indicated that both aequaline and schefferine contain a 9-methoxy substituent. In order to clarify this discrepancy and to establish unequivocally the correct structure of aequaline, compound **3** was synthesized by intramolecular Mannich condensation of (±)-norprotosinomenine (**18b**) with formaldehyde at pH 6.4 and room temperature. Cyclization occurred ortho and para to the phenolic hydroxyl group to give a mixture of (±)-3,9-dihydroxy-2,10-dimethoxytetrahydroprotoberberine (**3**) and (±)-3,11-dihydroxy-2,10-dimethoxytetrahydropseudoberberine (**5**). Spectroscopic comparison (IR, NMR, MS) of aequaline with compound **3** showed that aequaline did not have the structure assigned to it. Two diphenolic 2,3,9,10-substituted isomers of compounds **2** and **3** have been isolated from natural sources and are named discretamine (**6**)[11,12] and stepholidine (**7**).[13,14] Both compounds have recently been synthesized.[15] A comparison of aequaline with discretamine and stepholidine (IR, NMR, mass spectrometry, TLC) showed clearly that aequaline is identical with discretamine. It, therefore, also follows that schefferine must be 9-methoxy-10-hydroxy-substituted,

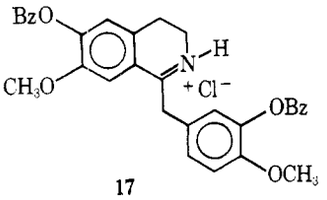
* Address correspondence to this author at University of California.



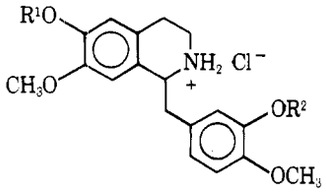
1, $R^1 = R^2 = R^4 = Me$; $R^3 = OMe$; $R^5 = R^6 = H$
2, $R^1 = R^4 = Me$; $R^3 = OH$; $R^2 = R^5 = R^6 = H$
3, $R^1 = R^5 = R^6 = H$; $R^3 = OH$; $R^2 = R^4 = Me$
4, $R^1 = R^2 = R^4 = Me$; $R^3 = OH$; $R^5 = R^6 = H$
5, $R^1 = R^3 = R^6 = H$; $R^2 = R^4 = Me$; $R^5 = OH$
6, $R^1 = R^4 = R^5 = R^6 = H$; $R^2 = Me$; $R^3 = OMe$
7, $R^1 = Me$; $R^2 = R^4 = R^5 = R^6 = H$; $R^3 = OMe$
8, $R^1 = R^2 = Me$; $R^3 = OMe$; $R^4 = R^5 = R^6 = H$
9, $R^1 = R^5 = H$; $R^2 = R^4 = R^6 = Me$; $R^3 = OMe$
10, $R^1 = R^2 = R^4 = Me$; $R^3 = R^6 = H$; $R^5 = OMe$
11, $R^1 = Me$; $R^2 = R^3 = R^4 = R^6 = H$; $R^5 = OMe$
12, $R^1 = R^3 = R^4 = R^6 = H$; $R^2 = Me$; $R^5 = OMe$
13, $R^1 = R^4 = Me$; $R^2 = R^3 = R^6 = H$; $R^5 = OH$
14, $R^1 = R^5 = R^6 = H$; $R^2 = R^4 = Me$; $R^5 = OMe$
15, $R^1 = R^4 = Me$; $R^2 = R^5 = R^6 = H$; $R^3 = OMe$



16



17

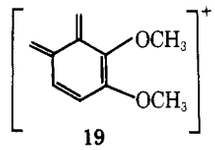


18a, $R^1 = R^2 = Bz$
b, $R^1 = R^2 = H$

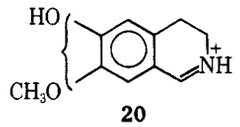
as indicated by preliminary mass spectroscopy,[10] giving it structure **8**. This compound was isolated by Cava et al.[13] from *Stephania glabra* as (−)-corydalmine and from *Corydalis pallida* by Kametani et al.,[16] who named it kikemanine. In 1962, Imaseki and Taguchi[17] isolated what they believed to be (+)-corydalmine from *Corydalis* species, but this compound was later shown to be identical with (±)-corybulbine[18] (**9**). (±)-Corydalmine [= (±)-kikemanine] has been synthesized.[19,20] Comparison (IR, NMR, MS, TLC) of schefferine with (±)-kikemanine showed that they have the same structure (**8**).

A protoberberine alkaloid believed to represent a new structure was isolated from *Corydalis pseudoadunca* by Yunosov et al.[21] and named coramine. Elemental analysis showed a $C_{19}H_{21}NO_4$ composition and methylation with diazomethane gave (−)-xylopinine (**10**), thus establishing a 2,3,10,11-tetraoxygenated substitution pattern. Based on degradative evidence structure **5** was proposed.[22] However, comparison of a sample of coramine with compound **5** obtained by synthesis (ir, NMR, mass spectrometry) showed that the two compounds were different. Coramine was also not identical with **11** and **12** produced by synthesis.[15] This left only (−)-coreximine (**13**) to be considered. Identity of coramine with coreximine was borne out by comparison of their respective IR, NMR, and mass spectra.

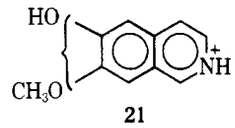
A tetrahydroprotoberberine alkaloid was isolated by Schmutz[11] in 1959 from *Xylopia discreta*. Elemental analysis showed a $C_{20}H_{23}NO_4$ composition and methylation with diazomethane gave (−)-tetrahydropalmatine. The alkaloid, which was named discretinine, was described as an isomer of corypalmine (**14**) and isocorypalmine (**15**), but no attempt was made to establish the position of the phenolic hydroxyl group. Mass spectrometry gave a molecular ion *m/e* 341, and the abundance of the $(M - OCH_3)^+$ fragment was 18% of the molecular ion peak, indicating the presence of a 9-methoxy substituent.[8] Fragment **19**, formed by retro-Diels–Alder cleavage of ring C, was the base peak at *m/e* 164. The second most abundant fragment had a mass *m/e* 149 (**19** − CH_3), while fragments **20** (*m/e* 178) and **21** (*m/e* 176) were considerably less prominent. This contrasts with tetrahydroprotoberberines containing a hydroxyl group in ring D (e.g., **2**–**8**), where the base peak is derived from the A/B moiety (fragments **20** and **22**). Having established a 9,10-dimethoxy substitution of ring D, there are only two alternative structures available for discretinine. It must be either corypalmine (**14**) or isocorypalmine (**15**). Spectroscopic comparison (IR, NMR) showed discretinine to be identical with corypalmine.



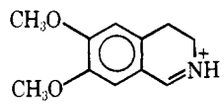
19



20



21



22

Experimental Section

General. Melting points (mp) were determined with a Thomas-Hoover apparatus and are uncorrected. Infrared (IR) spectra were obtained in potassium bromide, unless otherwise indicated, on a Perkin-Elmer 337 spectrometer. ^1H NMR spectra were obtained in deuteriochloroform with tetramethylsilane as an internal reference on a Varian XL-100 spectrometer equipped with a Nicolet Technology Corp. Fourier transform accessory. Electron impact (EI) mass spectra were taken on a AEI MS-12 mass spectrometer interfaced to a PDP 8/I computer using the DS-30 software.

(±)-Norprotosinomenine (18b). 3-Benzyloxy-4-methoxyphenethylamine[23] (3 g) and 3.2 g of 3-benzyloxy-4-methoxyphenylacetic[24] acid were mixed and fused at 160–170 °C for 4.5 h under reduced pressure. After cooling, the mixture was dissolved in 60 mL of chloroform and washed with 10% sodium bicarbonate solution, then with water, 10% hydrochloric acid, and, finally, with water again, and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a yellowish brown, oily substance (**16**), which crystallized from absolute ethanol (5.1 g): mp 114–115 °C (lit.[25] 113.5–115 °C); IR ν_{max}(Nujol) 3290 (NH), 1630 cm^{-1} (C=O). A mixture of 1 g of the amide (**16**), 1.1 mL of freshly distilled phosphorus oxychloride, and 15 mL of dry toluene was heated in an oil bath at 105–110 °C for 1.5 h in a nitrogen atmosphere. The reaction mixture was evaporated to dryness under reduced pressure. The residue was washed repeatedly with anhydrous benzene and dried to afford a brown, oily substance which crystallized from aqueous ethanol as light yellow needles (**17**): mp 145–148 °C (lit.[25] 145–148 °C); IR ν_{max}(Nujol) 2450 (br), 1850–1925 (immonium band), 1640 cm^{-1} (C=$^+$NH). The free base of **17** showed a band at

1653 cm^{-1} (C=N). The 1-benzyl-3,4-dihydroisoquinoline hydrochloride (**17**) (7.6 g) was dissolved in a mixture of 200 mL of methanol and 25 mL of water, and the solution cooled in ice water. Sodium borohydride (8 g) was added in small portions while stirring. After the addition was complete, the reaction mixture was stirred at room temperature for 10 min, then refluxed for 1 h. Removal of the solvent left a residue which was treated with water and extracted with chloroform. The combined chloroform extracts were washed with water and dried (Na_2SO_4) to give a pale yellow oil (4.2 g), which was converted to the hydrochloride (**18a**) and crystallized from methanol: mp 210–213 °C (lit. 207–210,[25] 206–208 °C[26]).

(±)-*O,O*-Dibenzyl-*N*-norprotosinomenine hydrochloride (**18a**) (1.9 g) was dissolved in 50 mL of ethanol and 70 mL of 25% hydrochloric acid and the solution heated under reflux for 1.5 h in a stream of nitrogen. Evaporation of the solvent left a residue which was dissolved in absolute ethanol and evaporated to dryness. This treatment with ethanol was repeated twice, and the residue was crystallized from methanol to give (±)-norprotosinomenine hydrochloride (**18b**): mp 236–242 °C (lit.[27] 241–242 °C). The IR (KBr) spectrum was identical with that of authentic (±)-norprotosinomenine.[28]

Mannich Reaction of (±)-Norprotosinomenine. (±)-Norprotosinomenine hydrochloride (**18b**) (0.5 g) was dissolved in 20 mL of methanol and 60 mL of water and the solution adjusted to pH 6.4 with 5% sodium bicarbonate solution. Formaldehyde solution (16 mL, 37%) was added and pH again adjusted to 6.4. After the reaction mixture had been kept at room temperature for 48 h, methanol was evaporated, water was added, and the solution was basified with sodium bicarbonate and extracted with chloroform. Evaporation of the solvent left a residue which was chromatographed on a column of neutral alumina[29] with chloroform to give 196 mg of (±)-3,9-dihydroxy-2,10-dimethoxytetrahydroprotoberberine (**3**). Crystallization from methanol afforded colorless prisms; mp 208–212 °C dec, after vacuum drying mp 218–221 °C dec; ir ν_{max}(KBr) 3350 (br), 2800–2700 (*trans*-quinolizidine); NMR δ ($CDCl_3$) 3.87 (3 H, s, OMe), 3.89 (3 H, s, OMe) 6.69 (2 H, s, ArH), 6.72 (2 H, s, ArH); MS (EI) *m/e* (rel intensities) 327 (72) (M^+), 326 (48), 178 (100), 176 (42), 150 (58), 135 (32). The abundance of the $(M - OCH_3)^+$ fragment was 2% of the molecular ion peak, indicative of the absence of a methoxyl group in position 9.[8] Gibb's reaction[30] was positive, showing an unsubstituted position para to the phenolic hydroxyl group. Anal. Calcd for $C_{19}H_{21}NO_4$: C, 69.70; H, 6.47; N, 4.30. Found: C, 69.68; H, 6.42; N, 4.30. Methylation with diazomethane gave (±)-tetrahydropalmatine (**1**), which crystallized from ether; mp 149–151 °C (lit.[31] 151–151.5 °C). IR spectrum was superimposable on that obtained with an authentic sample of (−)-tetrahydropalmatine.[32]

Elution of the column was continued with a mixture of chloroform and methanol (95:5) to yield (±)-3,11-dihydroxy-2,10-dimethoxytetrahydropseudoberberine (**5**) (119 mg). Crystallization from methanol afforded colorless prisms: mp 237–244 °C dec, after vacuum drying 251–255 °C dec (lit.[25] 232–235 °C); IR ν_{max}(KBr) 3500–3400 (br), 2800–2700 cm^{-1} (*trans*-quinolizidine); NMR δ ($CDCl_3$) 3.86 (3 H, s, OMe), 3.91 (3 H, s, OMe), 6.56 (1 H, s, ArH), 6.68 (1 H, s, ArH), 6.72 (2 H, s, ArH); MS (EI) *m/e* (rel intensities) 327 (69) (M^+), 326 (24), 178 (100), 176 (41), 150 (96), 135 (24). The $(M - OCH_3)^+$ fragment was 2% of the molecular ion peak and Gibb's reaction was negative. Anal. Calcd for $C_{19}H_{21}NO_4$: C, 69.70; H, 6.47; N, 4.30. Found: C, 69.43; H, 6.68; N, 4.18. Methylation of **5** (30 mg) with diazomethane gave (±)-xylopinine (**10**), which crystallized from ether as colorless needles: mp 145–147 °C (lit.[25] 146–148 °C); IR (KBr) spectrum was superimposable on that obtained with an authentic sample of (−)-xylopinine.[33]

(+)-Kikemanine [= (±)-corydalmine]. (±)-Stepholidine (**7**) (50 mg) was dissolved in 10 mL of methanol and treated with a solution of diazomethane in ether (prepared from 2 g of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide). After 45 min at room temperature the solution was evaporated to dryness. TLC of the residue on silica gel with chloroform–methanol (96:4) gave four spots, three of which were identified as unreacted stepholidine, isocorypalmine (**15**), and tetrahydropalmatine (**1**) by comparison with authentic substances. The fourth component of the mixture was isolated by preparative TLC on silica gel with chloroform–methanol (96:4) (double development) and crystallized from methanol; 11 mg; mp 166–168 °C dec (lit.[19] 187.5–188.5 °C corr); NMR δ ($CDCl_3$) 3.82 (3 H, s, OMe), 3.87 (3 H, s, OMe), 3.89 (3 H, s, OMe), 6.62 (1 H, s, ArH), 6.73 (1 H, s, ArH), 6.82 (2 H, s, ArH); MS (EI) *m/e* (rel intensities) 341 (66) (M^+), 340 (43), 310 (10), 192 (100), 190 (30), 150 (27), 135 (29). The $(M - OCH_3)^+$ was 15% of the molecular ion, indicative of a 9-methoxy substituent. The base peak *m/e* 192 (**22**) showed two methoxy groups in ring A. The isolated compound must, therefore, have structure **8**.

Aequaline. An authentic sample of aequaline exhibited proton resonances in $CDCl_3$ at δ 3.82 (3 H, s, OMe), 3.90 (3 H, s, OMe), 6.68 (1 H, s, ArH), 6.70 (1 H, s, ArH), 6.82 (2 H, s, ArH), identical with the NMR spectrum of (±)-discretamine,[15] but different from that of compound **3**. The MS (EI) of aequaline showed major peaks at *m/e* (rel intensities) 327 (52) (M^+), 326 (30), 296 (8.2), 178 (100), 176 (27), 150 (30), 135 (28). The $(M - OCH_3)^+$ fragment was 16% of the molecular ion peak, indicative of a 9-methoxy group[8] and in good agreement with that observed for natural[12] and synthetic[15] discretamine. R_f values (TLC) of aequaline were identical with those of (±)-discretamine, but different from those of compound **3** and stepholidine (**7**) on silica gel with (a) benzene–ethanol (92:8); (b) chloroform–methanol (96:4); and (c) ethyl acetate–methanol (96:4). The IR spectrum of aequaline was superimposable on that obtained with (±)-discretamine.

Schefferine. An authentic sample exhibited NMR peaks ($CDCl_3$) at δ 3.82 (3 H, s, OMe), 3.87 (3 H, s, OMe), 3.89 (3 H, s, OMe), 6.62 (1 H, s, ArH), 6.73 (1 H, s, ArH), and 6.82 (2 H, s, ArH), identical with those observed with (±)-kikemanine; MS (EI) *m/e* (rel intensities) 341 (66), 340 (43), 310 (10), 192 (100), 190 (29), 150 (26), 135 (25). The abundance of the $(M - OCH_3)^+$ fragment was 15% of the molecular ion peak, in good agreement with that observed for (±)-kikemanine. The R_f values of schefferine were identical with those of (±)-kikemanine on silica gel with (a) benzene–ethanol (92:8); (b) chloroform–methanol (96:4); and (c) ethyl acetate–methanol (96:4). The IR spectrum (KBr) of schefferine was superimposable on that of (±)-kikemanine.

Coramine. An authentic sample of coramine exhibited proton resonances ($CDCl_3$) at δ 3.85 (3 H, s, OMe), 3.87 (3 H, s, OMe), 6.54 (1 H, s, ArH), 6.58 (1 H, s, ArH), 6.69 (1 H, s, ArH), 6.81 (1 H, s, ArH); MS (EI) *m/e* (rel intensities) 327 (59) (M^+), 178 (100), 176 (28), 150 (81), 135 (17). The IR spectrum of coramine was different from that of compound **5**, but superimposable on the spectrum of (−)-coreximine.

Discretinine. MS (EI) of discretinine gave a molecular ion *m/e* (rel intensities) at 341 (64) and major fragments at 310 (12), 178 (8.2), 176 (22), 164 (100), 149 (59). The $(M - OCH_3)^+$ fragment was 18% of the molecular ion peak. NMR resonances of discretinine ($CDCl_3$) appeared at δ 3.85 (6 H, s, OMe), 3.89 (3 H, s, OMe), 6.68 (1 H, s, ArH), 6.70 (1 H, s, ArH), 6.81 (1 H, s, ArH), 6.84 (1 H, s, ArH), identical with those of authentic (±)-corypalmine. (−)-Isocorypalmine[34] showed the following proton resonances: δ 3.84 (6 H, s, OMe), 3.87 (3 H, s, OMe), 6.59 (1 H, s, ArH), 6.81 (2 H, s, ArH), 6.83 (1 H, s, ArH). The IR spectrum of discretinine was superimposable on that obtained with (±)-corypalmine.

Acknowledgments. We are grateful to the following colleagues for generous gifts of alkaloids used in this study: Professor E. Gellert, aequaline and schefferine; Professor R. H. F. Manske, coreximine; Professor M. Tomita, (±)-corypalmine; Dr. J. Schmutz, discretinine; Dr. M. S. Yunosov, coramine. This work was supported in part by the University of California Academic Senate Committee on Research and the NIH Division of Research Resources (RR 00892-1A1).

Registry No.—(±)-**1**, 2934-97-6; (±)-**3**, 62057-90-3; (±)-**5**, 214162-23-9; (±)-**6**, 55934-50-4; **6**, 1356-73-6; (±)-**7**, 16562-14-4; (±)-**8**, 32886-80-9; **8**, 30413-84-4; **10**, 13407-95-9; (±)-**13**, 6719-48-8; **13**, 483-45-4; (±)-**14**, 27313-86-6; **14**, 6018-40-2; **15**, 483-34-1; **16**, 21411-26-7; **17**, 37911-04-9; **17** free base, 21411-27-8; **18a**, 63511-81-9; **18b**, 19625-07-1; 3-benzyloxy-4-methoxyphenethylamide, 36455-21-7; 3-benzyloxy-4-methoxyphenylacetic acid, 5487-33-2.

References and Notes

(1) M. Shamma, "The Isoquinoline Alkaloids. Chemistry and Pharmacology", Academic Press, New York, N.Y., 1972, p 268.
(2) T. Kametani, "The Chemistry of the Isoquinoline Alkaloids", Elsevier, Amsterdam, 1969, p 109.
(3) R. H. F. Manske and W. R. Ashford in "The Alkaloids, Chemistry and Physiology", Vol. IV, R. H. F. Manske and Holmes Ed., Academic Press, New York, N.Y., 1954, p 77.
(4) P. W. Jeffs in ref 3, Vol. IX, 1967, p 41.
(5) D. H. R. Barton, R. H. Hesse, and G. W. Kirby, *Proc. Chem. Soc.*, 267 (1963); *J. Chem. Soc.*, 6379 (1965).
(6) A. R. Battersby, R. J. Francis, M. Hirst, and J. Staunton, *Proc. Chem. Soc.*, 268 (1963).
(7) E. Brochmann-Hanssen, C.-C. Fu, and G. Zanati, *J. Pharm. Sci.*, **60**, 873 (1971).
(8) W. J. Richter, and E. Brochmann-Hanssen, *Helv. Chim. Acta*, **58**, 203 (1975), and references cited therein.
(9) E. Gellert and R. Rudzats, *Aust. J. Chem.*, **25**, 2477 (1972).
(10) W. J. Richter and E. Brochmann-Hanssen, unpublished work.

(11) J. Schmutz, *Helv. Chim. Acta*, **42**, 335 (1959).
(12) W. J. Richter and E. Brochmann-Hanssen, *Helv. Chim. Acta*, **58**, 209 (1975).
(13) M. P. Cava, K. Nomura, S. K. Talapatra, M. J. Mitchell, R. H. Schlessinger, K. T. Buck, J. L. Beal, B. Douglas, R. F. Raffauf, and J. A. Weisbach, *J. Org. Chem.*, **33**, 2785 (1968).
(14) E. Brochmann-Hanssen and W. J. Richter, *J. Pharm. Sci.*, **64**, 1040 (1975).
(15) H.-C. Chiang and E. Brochmann-Hanssen, *J. Org. Chem.*, in press.
(16) T. Kametani, M. Ihara, and T. Honda, *J. Chem. Soc. C*, 1060 (1970).
(17) J. Imaseki and H. Taguchi, *J. Pharm. Soc. Jpn.*, **82**, 1214 (1962).
(18) H. Kaneko and S. Nanuto, *J. Org. Chem.*, **34**, 2803 (1969).
(19) S. A. Telang and C. K. Bradsher, *J. Org. Chem.*, **30**, 752 (1965).
(20) T. Kametani, T. Honda, and M. Ihara, *J. Chem. Soc. C*, 3318 (1971).
(21) M. S. Yunosov, S. T. Akramov, and S. Yu, *Dokl. Akad. Nauk SSSR*, **162**, 603 (1965); *Chem. Abstr.*, **63**, 5695 (1966).
(22) M. S. Yunosov, S. T. Akramov, and S. Yu, *Khim. Prir. Soedin.*, **2**, 340 (1966); *Chem. Abstr.*, **66**, 85906k (1967).
(23) A. R. Battersby, D. J. LeCount, S. Garratt, and R. I. Thrift, *Tetrahedron*, **14**, 46 (1961).
(24) A. R. Battersby, R. Binks, R. J. Francis, D. J. McCaldin, and H. Ramuz, *J. Chem. Soc.*, 3600 (1964).
(25) M. Tomita, and J. Kunimoto, *J. Pharm. Soc. Jpn.*, **80**, 1245 (1960).
(26) T. Kametani, R. Charubala, M. Ihara, M. Koizumi, K. Takahashi, and K. Fukumoto, *J. Chem. Soc. C*, 3315 (1971).
(27) D. H. R. Barton, R. James, G. W. Kirby, D. W. Turner, and D. A. Widdowson, *J. Chem. Soc. C*, 1529 (1968).
(28) E. Brochmann-Hanssen, C.-H. Chen, C. R. Chen, H.-C. Chiang, A. Y. Leung, and K. McMurtrey, *J. Chem. Soc., Perkin Trans. 1*, 1531 (1975).
(29) Aluminum oxide, Woelm, activity III.
(30) Spray solution: 1% 2,6-dichloroquinonechlorimide in ethanol, followed by exposure to ammonia vapors.
(31) T. Kametani and M. Ihara, *J. Chem. Soc. C*, 530 (1967).
(32) Pierce Chemical Co.
(33) Prepared by methylation of (−)-coreximine with diazomethane.
(34) Isolated from opium (Brochmann-Hanssen).

Structures of Steffimycin and Steffimycin B[1]

Robert C. Kelly,* Ilse Schletter, James M. Koert, Forrest A. MacKellar, and Paul F. Wiley*[2]

Research Laboratories, The Upjohn Company, Kalamazoo, Michigan 49001

Received April 26, 1977

A combination of chemical degradation and spectral studies has established that the structures of steffimycin and steffimycin B are those indicated by structures **1a** and **1b**, respectively.

The discovery of the antibiotic steffimycin (**1a**), produced by *Streptomyces steffisburgensis* and having activity against gram-positive organisms, was reported by Bergy and Reusser[3] some years ago. Subsequently, a description of the isolation of an antibiotic, steffimycin B (**1b**), having very similar physical, chemical, and biological properties, was published.[4] Brodasky and Reusser,[4] on the basis of private communications from Dr. R. C. Kelly, proposed a gross structure for steffimycin. Physical data of various kinds indicated that steffimycin and steffimycin B differed only by the presence of a methyl group in the latter which was absent in the former, and a structure was proposed for steffimycin B. However, the identity of the sugars present in these antibiotics was not published and very limited data were presented. The present paper proposes complete structures (**1a** and **1b**) for these antibiotics, except for stereochemistry in ring A of the linear tetracyclic system, and discusses the data on which these structures are based.

The original publication[3] on steffimycin established that it has a moleclar formula of $C_{28}H_{30}O_{13}$. The ultraviolet spectrum has maxima at 214, 236, 378, and 439 nm with the latter moving to 528 nm in base, which suggests that **1a** has a hydroxyanthraquinone chromophore[5-8] and is related to the anthracycline antibiotics.[9-11] It has been shown[5,6] that such a spectral pattern is present only in hydroxyanthraquinones having two hydroxyl groups α to the quinone carbonyl groups, and that these must be either 1,5 or 1,8. The infrared spectrum has bands at 1672 and 1620 cm^{-1}, which would be those expected for the hydrogen-bonded (1620 cm^{-1}) and nonbonded carbonyls of a 1,8-dihydroxyanthraquinone system.[12] Furthermore, the ^{13}C NMR spectrum of **1a** (Table I) has resonances at δ 179.3 and 189.1 which would arise from such an anthraquinone.[13] Conversion of the phenolic hydroxyls to methoxyls as in **1c** (see below) causes the downfield carbonyl resonance to shift to δ 181.4. In addition, an infrared band at 1710 cm^{-1} indicates a third carbonyl. The ^1H NMR (Me_2SO-d_6) spectrum of **1a** has chemical shifts of δ 6.75, 7.08, and 7.97 arising from aromatic protons present. Signals at δ 1.27 (d, 3 H) and 1.41 (s, 3 H) indicate two CH_3C groups with one being attached to a carbon bearing a proton. Singlets at δ 3.42, 3.44, and 3.90 can be assigned to CH_3O groups. Steffimycin B (**1b**) was found to have a molecular formula of $C_{29}H_{32}O_{13}$ and very similar spectra, except that one more CH_3O was present.[4] The data derived from **1a** and **1b** are so similar to those reported for aranciamycin[14] that it is clear that the three antibiotics are very closely related.

Acidic methanolysis of steffimycin gave rise to two products. One of these was a high-melting orange-red solid designated steffimycinone (**2a**), and the other was a colorless syrup (**4a**) characterized as a diacetate (**4b**), a mono-*p*-nitrobenzoate (**4c**), and a di-*p*-nitrobenzoate (**4d**). Methanolysis of steffimycin B also gave two products. One of these was shown to be **2a** by comparison of physical properties. The second was a second colorless syrup (**4e**), which differed from **4a.** Compound **2a** was shown by analysis and mass spectrometry to have a molecular formula of $C_{21}H_{18}O_9$. Its ultraviolet and infrared spectra were very similar to those of **1a** and were consistent with the assignment of a 1,8-dihydroxyanthraquinone structure to which was attached an aliphatic moiety containing a carbonyl group. The ^{13}C and ^1H NMR spectra indicated that three aromatic protons as well as one of the CH_3C groups and two of the methoxyl groups were present, one of which was attached to an aromatic ring (s, 3 H, δ 3.90) and one to an aliphatic system (s, 3 H, δ 3.48). The resonance arising from the CH_3C was a singlet, indicating the absence of a proton adjacent to the methyl protons. Doublets at δ 3.62 and 5.24 with coupling constants at 3.0 Hz represented 2 H which must be on adjacent carbon atoms. The molecular formula of **2a** accounts for all but a $C_7H_{12}O_4$ moiety of **1a,** which would suggest that **2a** is formed by methanolysis of **1a** to form an aglycone (**2a**) and a sugar (**4a**), which would have a molecular formula of $C_7H_{14}O_5$.

Catalytic reduction of **1a** under low pressure resulted in isolation of a new compound, **2b.** This material was very