protons in lanuginosine show a 1,2,4 pattern with the same coupling constants as those for oxoxylopine, although the chemical shifts are significantly different (see Table I). In view of the considerations discussed above, it appears likely that lanuginosine is the 10-methoxy isomer of xylopine and should be represented by structure **4** rather than structure **1**. It is noteworthy that michepressine iodide (**7**), an aporphine corresponding in substitution pattern to structure **4**, has been isolated from *Michelia compressa*.[15] Since oxoaporphine alkaloids are probably formed in the plant *via* oxidation of the corresponding aporphines,[16,17] the isolation of michepressine iodide from a *Michelia* species supports assignment of the revised structure **4** for lanuginosine.

Experimental Section

Melting points were determined on a Thomas–Hoover Unimelt apparatus and are corrected. Ir spectra were determined on a Beckman IR-9 double-beam recording spectrophotometer. Uv spectra were determined on a Beckman DK-2A recording spectrophotometer. Nmr spectra were determined on a Varian Associates A-60A spectrometer. Microanalyses were performed by Spang Microanalytical Laboratories, Ann Arbor, Mich. Mass spectra were measured on a Hitachi RMU-6A spectrometer. We thank the Purdue Mass Spectrometry Center, supported under U. S. Public Health Service Grant FR-00354, for the mass spectral data.

Extraction and Preliminary Fractionation.—The dried ground roots and rhizomes (7 kg) of *Stephania abyssinica* were extracted continuously with ethanol until the extract returning to the pot was nearly colorless. Evaporation of the ethanolic extract gave a mobile semisolid residue (901 g) which was triturated three times with 1.6 *N* hydrochloric acid (3-l. total) to leave a gummy residue (143 g). The aqueous solution was partially basified with concentrated ammonium hydroxide solution to pH 5 and extracted with chloroform (four 1-l. portions) to yield, after evaporation, the weak base fraction (28.7 g). The remaining aqueous solution was decanted from insoluble residue (70 g), basified to pH 8 with concentrated ammonium hydroxide solution, and extracted with chloroform (four 1-l. portions) to give, after evaporation, the strong base fraction (10.4 g).

Oxoxylopine (**1**).—The weak base fraction was chromatographed over silicic acid (900 g), eluting with chloroform, 1% methanol–chloroform, 2.5% methanol–chloroform, and 5% methanol–chloroform. The fraction eluted with 2.5% methanol–chloroform (6 g) was rechromatographed over acid-washed alumina, eluting with benzene–chloroform mixtures. A fraction eluted with 2:1 benzene–chloroform (150 mg) crystallized on standing to give orange prisms (108 mg). Two recrystallizations from chloroform yielded oxoxylopine (**1**, 70 mg): mp 319–321° dec; $\lambda_{max}^{CHCl_3}$ 246 mμ (ε 28,650), 271 (21,800), 314 (5960); $\lambda_{max}^{0.1\,N\,HCl}$ 257 mμ (ε 20,570), 284 (15,500); λ_{max}^{KBr} 3.37, 6.02, 6.24, 6.36, 6.67, 6.86, 7.06, 7.66, 7.92, 8.17, 9.57, 9.84 μ; *m/e* 305 (M^+, 100%), 275 ($M^+ - CH_2O$, 15%).

Anal. Calcd for $C_{18}H_{11}NO_4$: C, 70.81; H, 3.63; N, 4.59. Found: C, 70.88; H, 3.76; N, 4.63.

Oxoxylopine was found to be nearly insoluble in ethanol, methanol, benzene, ethyl acetate, ether, cyclohexane, and acetone, and only sparingly soluble in chloroform. Its chloroform solution exhibited a strong green-yellow fluorescence in visible light. Oxoxylopine showed a cherry-red coloration upon treatment with dilute hydrochloric or sulfuric acid, as observed earlier for other oxoaporphine alkaloids.

Conversion of Oxoxylopine (**1**) **into** (±)-**N-Acetylxylopine** (**6**).—A solution of oxoxylopine (22 mg) in acetic acid–water (2:1, 2 ml) was treated with powdered zinc (3 g) and 10 *N* hydochloric acid (6 ml). The reaction mixture was heated with stirring at 100° for 18 hr, after which time the zinc had been consumed and the reaction mixture turned red, indicating the presence of unreduced oxoaporphine. Additional zinc dust (1 g) and concentrated hydrochloric acid (3 ml) was added and the reaction was stirred at 100° for an additional 24 hr, after which time the zinc had again been consumed and the reaction mixture was colorless. The acidic solution was made strongly basic with a large excess of concentrated ammonium hydroxide solution and was extracted with chloroform (five 150-ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to give crude (±)-xylopine (18 mg; λ_{max}^{MeOH} 217, 237, 280, 320 mμ) (*cf.* 12), which was acetylated without further purification. Treatment of the (±)-xylopine with acetic anhydride (1 ml) and pyridine (1 ml) at 70° for 0.5 hr, followed by standing at room temperature for an additional 3 hr and evaporation under reduced pressure, gave a light brown gummy residue. This material was dissolved in ether–chloroform (3:1, 50 ml) and washed successively with 50 ml of 0.5 *N* hydrochloric acid, 1 *N* sodium hydroxide, and water. The organic phase was dried (Na_2SO_4) and the solvent was evaporated to give a residue which was crystallized twice from acetone–ether to give colorless needles (9 mg), mp 216–218°. The product was chromatographed over silicic acid (5 g) in chloroform to give (±)-N-acetylxylopine (5 mg): λ_{max}^{EtOH} 216.5 mμ (ε 32,200), 283 (16,700); $\lambda_{max}^{CHCl_3}$ 3.32, 3.41, 3.45, 3.52, 6.12, 6.33 μ. The spectra were indistinguishable from those of an authentic sample.

Registry No.—**1**, 23740-25-2; **2**, 475-75-2; **3**, 3912-57-0; **6**, 23740-28-5.

(15) K. Ito, *J. Pharm. Soc. Jap.*, **81**, 703 (1961).

(16) J. Cohen, W. Von Langenthal, and W. I. Taylor, *J. Org. Chem.*, **26**, 4143 (1961).

(17) NOTE ADDED IN PROOF.—Subsequent to submission of the manuscript, Dr. A. J. Liepa has isolated and characterized xylopine from the weak base fraction from *S. abyssinica*.

Selective O-Demethylation of Papaverine[1]

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Prior art has shown that papaverine (**1**) can be O-demethylated partially to the diphenol **6** by refluxing concentrated HCl[2] and completely to the tetraphenol papaveroline (**8**) by refluxing 48% HBr.[3] In connection with our interest in the partial O demethylation of polymethoxylated alkaloids,[4] we investigated the acid-catalyzed ether cleavage of **1** in more detail.

Thin layer chromatography using authentic samples of the various phenols as standards provided an excellent tool for this purpose. The monophenols **2**–**5** and papaveroline (**8**) were prepared according to literature procedures,[3,5] whereas the diphenol **6** and the triphenol **7** were synthesized by the conventional methods outlined in Schemes I and II, respectively. Analysis of the reaction mixture obtained by refluxing papaverine (**1**) with concentrated HCl for several hours showed the presence of starting material and the five phenols **2**, **3**, **6**, **7**, and **8**. The two monophenols **4** and **5** were not detected. The major component in this reaction mixture proved to be the diphenol **6**, which could be isolated in good yield but whose physical properties differed considerably from those reported.[2]

The interrelationship of the cleavage products follows. Treatment of papaverine (**1**) with liquid HBr gave a 1:1 mixture of the two monophenols **2** and **3**, as

(1) Presented in part by A. Brossi at the 13th Symposium on the Chemistry of Natural Products, Sapporo, Japan, Sept 25–27, 1969, Symposium Papers, pp 177–186.

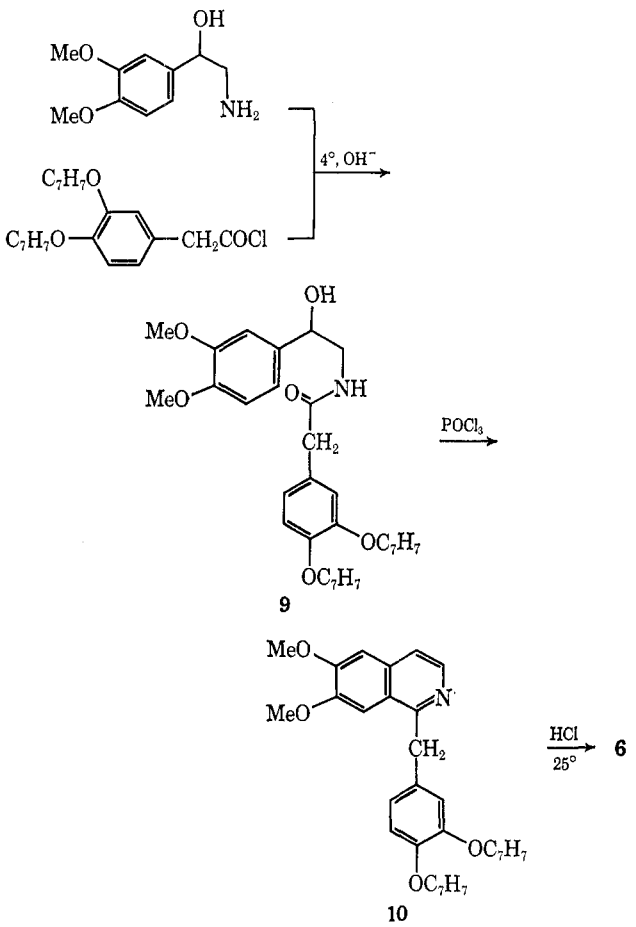
(2) A. Boucherle and J. Alary, *Bull. Soc. Chim. Fr.*, 1222 (1960).

(3) J. V. Burba and M. F. Murnaghan, *Biochem. Pharm.*, **14**, 823 (1965).

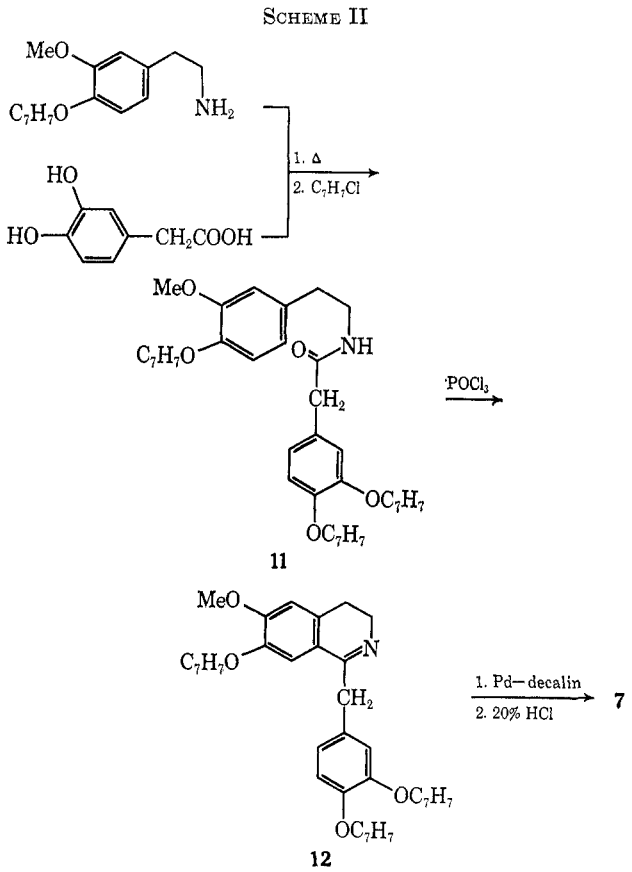
(4) A. Brossi and S. Teitel, *Helv. Chim. Acta*, **52**, 1228 (1969).

(5) E. Brochmann-Hanssen and K. Hirai, *J. Pharm. Sci.*, **57**, 940 (1968).

SCHEME I

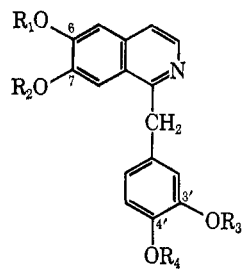


SCHEME II



shown by nmr and glpc. Short-time treatment of **1** with refluxing 48% HBr gave the diphenol **6** in more than 40% yield, whereas a longer reflux period provided the triphenol **7** in more than 80% yield. The mixture of the monophenols **2** and **3** was transformed stepwise with 48% HBr to afford the diphenol **6**, then the triphenol **7**, and finally the tetraphenol **8**.

Thus our studies show that the course of O-demethylation of papaverine (**1**) with mineral acid involves first the two methoxy groups in the benzylic side chain to provide the mixture of monophenols **2** and **3** and the diphenol **6**, then the methoxy group in the 7 position to form the triphenol **7**, and finally the 6-methoxyl[6] to give papaveroline (**8**). These cleavage conditions also provide a facile route to the preparation of these compounds.

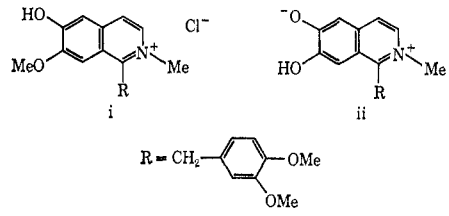


	R_1	R_2	R_3	R_4
1	Me	Me	Me	Me
2	Me	Me	Me	H
3	Me	Me	H	Me
4	Me	H	Me	Me
5	H	Me	Me	Me
6	Me	Me	H	H
7	Me	H	H	H
8	H	H	H	H

Experimental Section[7]

Mixture of 6,7-Dimethoxy-1-(4-hydroxy-3-methoxybenzyl)isoquinoline (2) and 6,7-Dimethoxy-1-(3-hydroxy-4-methoxybenzyl)-isoquinoline (3).—To 200 ml of liquid HBr at −78° was added a solution of 5 g (14.8 mmol) of papaverine (**1**) in 500 ml of CH_2Cl_2. The mixture was allowed to warm to room temperature and stored for 72 hr and the solvent was evaporated. The residue was dissolved in water, rendered alkaline with 5% NaOH, and extracted with EtOAc. The organic extract was evaporated to give 3.5 g (65%) of **1**. The alkaline aqueous layer was filtered free of solids, adjusted to pH 8 with 20% HCl, and extracted with $CHCl_3$. The extract was concentrated to a low volume and stored at 4° and the tan crystals were filtered to give 1.5 g (82% overall) of a 1:1 mixture of **2** and **3**: mp 160–162°, identical with the melting point of a 1:1 authentic mixture of **2**[5] and **3**;[5] R_f 0.74, identical with the R_f of authentic **2**[5] and **3**[5] (R_f of **4**[5] 0.66, R_f of **5**[5] 0.29); nmr ($CDCl_3$) δ 3.70, 3.82, 3.88, 3.99 (6 CH_3O), 4.50 (broad CH_2), 6.77,6.80 ($C_{2'}$,$C_{5'}$, $C_{6'}$), 7.04 (C_5 or C_8), 7.31 (multiplet, C_4 and C_5 or C_8), 8.16, 8.30 (C_3) [nmr of authentic

(6) The relative resistance of the 6- and 7-methoxyls in **1** to cleavage with mineral acid is in marked contrast to their lability on thermal fusion of **1** HCl which affords a mixture of the protopapaverines i and ii; see B. K. Cassels and V. Deulofeu, *Tetrahedron, Suppl.* **8**, part II, 485 (1966), and references cited therein.



(7) Melting points were taken on a Thomas–Hoover melting point apparatus and are corrected. Thin layer chromatography employed silica gel G plates developed for 10 cm with ethyl acetate–methanol–concentrated ammonium hydroxide (95:5:5) and detected with Dragendorff's reagent. The ir spectra were determined with either a Beckman IR-9 or a Perkin-Elmer 621 recording spectrophotometer and the uv spectra with a Cary 14 spectrophotometer using ethanol as solvent unless otherwise noted. The nmr spectra were obtained with either a Jeolco C-60H or a Varian HA-100 spectrometer using DMSO-d_6 as solvent except as noted and tetramethylsilane as internal standard. Gas–liquid partition chromatography (glpc) was done on a Barber–Colman Model 5000 instrument at 230° on a 200 cm × 2 mm i.d. glass column packed with Corning GLC 110 glass beads coated with 0.4% OV-101 (Applied Science Laboratories, Inc.). Extracts of products were washed with water and dried over anhydrous sodium sulfate prior to evaporation.

2—δ 3.70, 3.87, 3.96 (3 CH_3O), 4.49 (CH_2), 6.76, 6.79 ($C_{2'}$, $C_{5'}$, $C_{6'}$), 7.02, 7.34 (C_5, C_8), 7.40 (C_4), 8.34 (C_3); nmr of authentic 3—δ 3.82, 3.89, 3.99 (3 CH_3O), 4.49 (CH_2), 6.78 ($C_{2'}$, $C_{5'}$, $C_{6'}$), 7.04, 7.33 (C_5, C_8), 7.35 (C_4), 8.18 (C_3)]; glpc of TMS derivative of mixture of **2** and **3** gave Kovats Retention Indices of 2800 and 2830 corresponding to the values obtained from the TMS derivative of authentic **3** and **2**, respectively.

Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89. Found: C, 69.79; H, 6.06.

6,7-Dimethoxy-1-(3,4-dihydroxybenzyl)isoquinoline (6). A. By Synthesis.—To a solution of 11.2 g (32 mmol) of 3,4-dibenzyloxyphenylacetic acid[8] in 150 ml of $CHCl_3$ was added 1.55 ml of $SOCl_2$. The mixture was stirred and refluxed for 2 hr and evaporated *in vacuo*. The residue was dissolved in 50 ml of CH_2Cl_2 and added over 30 min to a vigorously stirred mixture of 6.3 g (32 mmol) of 2-(3,4-dimethoxyphenyl)-2-hydroxyethylamine[9] in 50 ml of CH_2Cl_2 and 10 ml of water, maintained at 4° and slightly alkaline by the addition of 10% NaOH as needed. The mixture was stirred at 25° for 1 hr, the organic layer was separated and evaporated, and the residue was crystallized from Et_2O to give 13.6 g (80%) of N-(3,4-dimethoxy-β-hydroxyphenethyl)-2-(3,4-dibenzyloxyphenyl)acetamide (**9**): mp 116–117°; ir ($CHCl_3$) 3610, 3420, 1655, 1510, 1260 cm^{-1}.

Anal. Calcd for $C_{32}H_{33}NO_6$: C, 72.84; H, 6.30. Found: C, 72.72; H, 6.11.

A mixture of 10.6 g (20 mmol) of **9** and 20 ml of $POCl_3$ in 120 ml of toluene was refluxed for 2 hr and evaporated *in vacuo*. A solution of the residue in water was rendered alkaline with 10% NaOH and extracted with EtOAc. The extract was evaporated and the residue was crystallized from EtOAc to give 9.4 g (96%) of 1-(3,4-dibenzyloxybenzyl)-6,7-dimethoxyisoquinoline (**10**): mp 120–121°; uv max 240 mμ (ε 66,100), 269 (6600) (sh), 279 (7000), 290 (5250) (sh), 314 (3750), 327 (4500); nmr δ 3.83, 3.92 (2 CH_3O), 4.47 (CH_2), 5.04 (2 CH_2O), 6.8–7.7 (16 aromatics), 8.24 (C_3 or C_4).

Anal. Calcd for $C_{32}H_{29}NO_4$: C, 78.18; H, 5.95. Found: C, 78.23; H, 5.81.

A mixture of 2.45 g (5 mmol) of **10**, 20 ml of concentrated HCl and 20 ml of C_6H_6 was vigorously stirred at 25° for 17 hr and evaporated *in vacuo*. The residue was dissolved in water and rendered alkaline with 5 ml of concentrated NH_4OH. The precipitate that formed was filtered and crystallized from MeOH to give 1.2 g (78%) of **6**: mp 199–200°; R_f 0.52; uv max 236 mμ (ε 62,900), 267 (5800) (sh), 278 (6500), 280 (5400) (sh), 312 (3850), 325 (4470); nmr δ 3.88, 3.92 (2 CH_3O), 4.37 (CH_2), 6.63 ($C_{2'}$, $C_{5'}$, $C_{6'}$), 7.28, 7.47 (C_5, C_8), 7.54 (C_4), 8.23 (C_3), 8.40, 8.64 (2 OH).

Anal. Calcd for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.50. Found: C, 69.55; H, 5.70.

An aliquot of **6** was converted into the hydrochloride and crystallized from EtOH: mp 232–233°; ir (KBr) 3380, 3240–2600, 1640, 1615, 1520, 1290 cm^{-1}; uv max 227 mμ (ε 25,400) (sh), 253 (55,200), 288 (6800), 311 (8300), 327 (6600) (sh), 345 (5000) (sh); uv max (0.1 *N* KOH) 233 mμ (ε 41,700), 270 (16,500) (sh), 324 (9600), 390 (7500) (sh); nmr δ 3.97, 3.99 (2 CH_3O), 4.76 (CH_2), 6.6–6.8 ($C_{2'}$, $C_{5'}$, $C_{6'}$), 7.64, 7.77 (C_5, C_8), 8.18, 8.30 (C_3, C_4), 8.88 (broad, OH, NH^+).

Anal. Calcd for $C_{18}H_{17}NO_4 \cdot HCl$: C, 62.16; H, 5.22. Found: C, 62.27; H, 5.39.

An aliquot of **6** was converted into the picrate and crystallized from MeOH: mp 180–181°, uv max 238 mμ (ε 61,400), 254 (28,400) (sh), 281 (7600), 292 (7400) (sh), 314 (9500) (sh), 327 (12,750), 353 (15,600), 415 (8200) (sh); nmr δ 4.00, 4.03 (2 CH_3O), 4.70 (CH_2), 6.62 ($C_{2'}$, $C_{5'}$, $C_{6'}$), 7.68, 7.80 (C_5, C_8), 8.15, 8.40 (C_3, C_4), 8.56 (picric acid aromatics).

Anal. Calcd for $C_{18}H_{17}NO_4 \cdot C_6H_3N_3O_7$: C, 53.34; H, 3.73. Found: C, 53.23; H, 3.88.

B. From 1 and Concentrated HCl.—A solution of 7 g (18.7 mmol) of **1** HCl in 30 ml of concentrated HCl was refluxed for 9 hr and cooled to 25°. The supernatent was decanted from the resulting oil and the latter was crystallized from Me_2CO to give 1 g of a mixture: mp 190–206°; R_f 0.85, 0.74, 0.52, 0.24, and 0.04 corresponding to values exhibited by **1**, **2** and **3**, **6**, **7**, and **8**. The Me_2CO mother liquors were evaporated; the residue was dissolved in 20 ml of water and rendered alkaline with 5 ml of concentrated NH_4OH. The precipitate that formed was filtered and crystallized from MeOH to give 3.7 g (64%) of **6**, mp 199–200°, identical in mixture melting point, tlc, and uv and nmr spectroscopy with **6** prepared *via* A. An aliquot was converted into the hydrochloride, mp 230–232° (lit.[2] mp 170°[10]), identical in melting point and tlc with **6** HCl prepared *via* A. An aliquot, when converted into the picrate, melted at 180–182° (lit.[11] mp 104°[10]) and caused no mixture melting point depression on admixture with the picrate of **6** prepared *via* A.

C. From 1 and 48% HBr.—A solution of 5 g (14.8 mmol) of papaverine (**1**) in 50 ml of 48% HBr was refluxed for 10 min, cooled, and diluted with 100 ml of water; the pH was adjusted to 8 with concentrated NH_4OH. The gum that precipitated solidified on standing and was filtered and washed with water. The solid was suspended in 40 ml of MeOH and refluxed for 1 hr. The resulting crystals were filtered and dried to give 2.16 g (47%) of **6**, mp 199–200°, identical in mixture melting point and tlc with **6** prepared *via* A.

D. From a Mixture of 2 and 3 and 48% HBr.—A solution of 1 g (3.1 mmol) of the 1:1 mixture of **2** and **3** in 10 ml of 48% HBr was refluxed for 6 min and worked up by the procedure given in C to yield 400 mg (42%) of **6**, mp 200–201°, identical in mixture melting point and tlc with **6** prepared *via* A.

1-(3,4-Dihydroxybenzyl)-7-hydroxy-6-methoxyisoquinoline (7). A. By Synthesis.—A mixture of 21 g (0.125 mol) of 3,4-dihydroxyphenylacetic acid and 32 g (0.125 mol) of 2-(4-benzyloxy-3-methoxyphenyl)ethylamine[12] was heated under N_2 at 180–190° for 2 hr, cooled, and dissolved in 1 l. of EtOH. To the solution was added 84 g (0.665 mol) of benzyl chloride and 91 g (0.665 mol) of anhydrous K_2CO_3. The mixture was stirred and refluxed for 20 hr and filtered hot. The filtrate was cooled and the resulting crystals were filtered and recrystallized from EtOH to give 52 g (71%) of N-(4-benzyloxy-3-methoxyphenethyl)-2-(3,4-dibenzyloxyphenyl)acetamide (**11**): mp 125–126°; ir ($CHCl_3$) 3440, 1670, 1525, 1265 cm^{-1}.

Anal. Calcd for $C_{38}H_{37}NO_5$: C, 77.60; H, 6.35. Found: C, 77.35; H, 6.55.

A mixture of 30 g (0.051 mol) of **11** and 60 ml of $POCl_3$ in 600 ml of toluene was refluxed for 2 hr and evaporated *in vacuo*. The residue was dissolved in water, EtOAc was added, and the mixture was rendered alkaline with 20% NaOH. The organic extract was acidified with ethanolic HCl and evaporated. The residue was twice crystallized from a mixture of EtOH and Et_2O to give 22.4 g (73%) of 1-(3,4-dibenzyloxybenzyl)-7-benzyloxy-6-methoxy-3,5-dihydroisoquinoline hydrochloride (**12** HCl): mp 193–194°; ir (KBr) 3000–2400, 1650, 1610, 1520, 1270 cm^{-1}; uv max 236 mμ (ε 18,600) (sh), 246 (17,400) (sh), 253 (14,700) (sh), 277 (5300) (sh), 309 (8400), 362 (7500).

Anal. Calcd for $C_{38}H_{35}NO_4 \cdot HCl$: C, 75.30; H, 5.99. Found: C, 75.31; H, 6.26.

A solution of 6 g (0.01 mol) of **12** HCl was dissolved in water, rendered alkaline with 10% NaOH, and extracted with EtOAc and the extract was evaporated. The residue was dissolved in 150 ml of decalin, 3 g of 10% Pd–C was added, the mixture was stirred and refluxed for 3 hr and filtered, and the filtrate was evaporated. The residual oil (4.5 g) was dissolved in 170 ml of 20% HCl, refluxed for 2 hr, and evaporated *in vacuo*. The residue was crystallized from EtOH and recrystallized from a mixture of MeOH and Et_2O to give 1.5 g (51%) of **7** HCl: mp 250–251°; R_f (0.24); uv max 237 mμ (ε 37,250), 281 (4400), 290 (4000) (sh), 313 (4000), 328 (3500), 353 (2200) (sh); nmr δ 4.04 (CH_3O), 4.94 (CH_2), 7.2 ($C_{2'}$, $C_{5'}$, $C_{6'}$), 7.67, 7.79 (C_5, C_8), 8.08, 8.28 (C_3, C_4), 8.80 (broad, OH), 10.94 (NH^+).

Anal. Calcd for $C_{17}H_{15}NO_4 \cdot HCl$: C, 60.99; H, 4.85. Found: C, 60.66; H, 5.18.

An aliquot of **7** HCl was dissolved in water and rendered alkaline with NH_4OH. The precipitate that formed was filtered (darkened on standing), dissolved immediately in EtOH, rendered acidic with ethanolic HBr, and evaporated. The residue was crystallized from EtOH to give **7** HBr, mp 245–246°.

Anal. Calcd for $C_{17}H_{15}NO_4 \cdot HBr$: C, 53.95; H, 4.28. Found: C, 53.88; H, 4.65.

B. From 1 and 48% HBr.—A solution of 1 g (2.96 mmol) of **1** in 10 ml of 48% HBr was refluxed for 1 hr and evaporated *in vacuo*. The residual solid was crystallized from a mixture of MeOH and Et_2O to give 900 mg (82%) of **7** HBr, mp 246–248°, identical in mixture melting point, tlc, and nmr spectroscopy with the **7** HBr prepared *via* A.

(8) T. Kametani and S. Kano, *J. Pharm. Soc. Jap.*, **85**, 256 (1965).

(9) N. A. Chaudhury and A. Chatterjee, *J. Indian Chem. Soc.*, **36**, 585 (1959).

(10) Obtained by cleavage of papaverine and probably contaminated.

(11) A. Pictet and G. H. Kramers, *Arch. Sci. Phys. Na. Genève*, **15**, 124 (1903) [*Chem. Zentr.*, **1**, 844 (1903)].

(12) J. M. Bobbitt and T-T. Chou, *J. Org. Chem.*, **24**, 1106 (1959).

C. From 6 and 48% HBr.—A solution of 1 g (3.22 mmol) of 6 in 10 ml of 48% HBr was refluxed for 30 min, diluted with 10 ml of water, and stored at 4°. The crystals that formed were filtered, washed with water, and dried to give 1 g (90%) of 7 HBr, mp 246–248°, identical in mixture melting point and tlc with the 7 HBr prepared *via* A.

1-(3,4-Dihydroxybenzyl)-6,7-dihydroxyisoquinoline Hydrobromide (8 HBr).—A solution of 2 g (5.9 mmol) of 7 HBr in 20 ml of 48% HBr was refluxed for 8 hr and evaporated *in vacuo*. The residue was crystallized from water to give 1.42 g (74%) of 8 HBr (identical in mixture melting point and tlc with the 8 HBr, obtained from 1 in 80% yield by the same procedure[4]): mp 257–259°; R_f 0.04; nmr δ 4.50 (CH_2), 6.60 ($C_{2'}$, $C_{5'}$, $C_{6'}$), 7.40, 7.65 (C_5, C_8), 8.00, 8.23 (C_3, C_4), 9.00 (broad, OH, NH^+).

Anal. Calcd for $C_{16}H_{13}NO_4 \cdot HBr$: C, 52.75; H, 3.87. Found: C, 52.81; H, 4.01.

Registry No.—1, 58-74-2; 2, 18813-60-0; 3, 18694-10-5; 6, 16637-56-2; 6 HCl, 16637-68-6; 6 picrate, 23740-72-9; 7 HCl, 23829-46-1; 7 HBr, 23740-73-0; 8 HBr, 23740-74-1; 9, 23740-75-2; 10, 23740-76-3; 11, 4672-97-3; 12 HCl, 4761-17-5.

Acknowledgment.—We are indebted to our Physical Chemistry Department under the direction of Dr. P. Bommer for the spectral and microanalytical data. We are grateful to Mr. J. O'Brien for technical assistance and to Professor G. Büchi, Massachusetts Institute of Technology, for fruitful discussions.

Microbiological Hydroxylation of Allethrone

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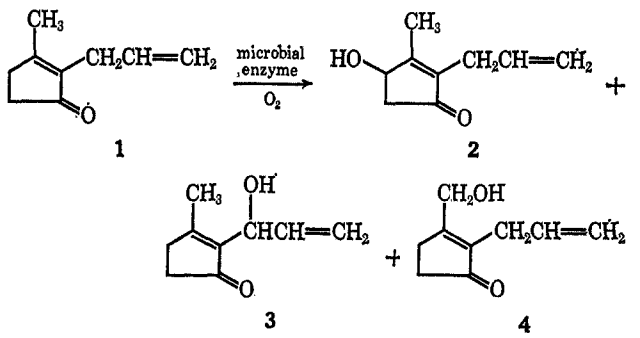
A number of synthetic analogs of the natural pyrethrins[1] exhibit high insecticidal activity. One of these, allethrin,[2] is prepared commercially by esterification of allethrolone (2) with chrysanthemic acid. We have recently investigated the microbiological conversion of cinerone into cinerolone[3] and now wish to report a similar conversion of allethrone (1) into allethrolone (2). Allethrone (1) was prepared by treatment of 2-N-pyrrolidino-5-methyl-2-cyclopenten-1-one with allylmagnesium bromide followed by dehydration as described by Dahill.[4]

Incubation of 1 with *Aspergillus niger* NRRL 3228 for 12 days gave a crude product, which was shown by glpc analysis to contain three major components (retention times of 5.5, 6.6, and 11.6 min, respectively). Small amounts of the three pure components were separated by preparative glpc and their mass spectra showed them to be monohydroxylated isomers of allethrone. Based on allethrone consumed, the yield of the mixture was 22%. The major component (73% of the mixture) was identified as allethrolone (2) by comparison of its glpc retention time and spectra (ir, nmr, and mass) with those of the authentic compound.[5]

The component with the shortest retention time (5% of the mixture) gave an infrared spectrum with a carbonyl band at 5.95 μ, indicating hydrogen bonding to the ketone. The nmr spectrum revealed three vinyl protons, one ring methyl, and two ring methylenes. The nmr absorption of the side-chain methylene group was absent and was replaced by a broad peak at δ 5.06. These data are consistent with structure 3, which incorporates a side-chain hydroxyl group.

The component with the longest retention time (18% of the mixture) gave a normal cyclopentenone carbonyl band at 5.88 μ in the infrared spectrum. The nmr spectrum exhibited three vinyl protons, two ring methylenes, and a side-chain methylene. The nmr absorption of the ring methyl was absent and a two-proton singlet appeared at δ 4.50 consistent with structure 4, in which hydroxylation has occurred on the ring methyl group.



Experimental Section[6]

Thirty 500-ml erlenmeyer flasks containing 100-ml quantities of fermentation medium were inoculated with a heavy filamentous growth of *Aspergillus niger* NRRL 3228 and incubated at 28° on a rotary shaker. The shaker was operated at 280 rpm and described a 2-in. circular orbit. After 2 days, 50 mg of allethrone (1) dissolved in 2 ml of absolute ethanol was added to each flask. Incubation was continued for 12 days and the contents of the flasks were then pooled and filtered to remove the cells. The cells were washed with 300 ml of distilled water and the washing was added to the filtrate. The filtrate was extracted with three equal volume portions of methylene chloride. After drying (Na_2SO_4), the solvent was removed at reduced pressure and the residual oil was distilled. The low-boiling fraction [bp 35–47° (0.1 mm), 0.49 g] was shown by thin layer chromatography (tlc) to contain only unreacted allethrone. The high-boiling fraction [bp 47–110° (0.1 mm), 0.55 g] on tlc revealed a spot with an identical R_f as authentic allethrone along with a slower moving spot with an identical R_f as authentic allethrolone. The allethrone (0.13 g) was separated from the allethrolone fraction (0.24 g) by preparative tlc on silica gel. Evaporative distillation of the allethrolone fraction at 0.1 mm (bath temperature 120°) gave 0.22 g of a colorless oil, λ_{max}^{EtOH} 229 mμ (ε 11,300). Glpc on a 10% EPON column at 200° showed three major peaks: peak 1, structure 3 (5% of the total, retention time of 5.5 min); peak 2, structure 2 (73% of the total, retention time of 6.6 min, identical with the retention time of authentic allethrolone); and peak 3, structure 4 (18% of the total, retention time of 11.6 min). Mass spectra were obtained using a Finnigan mass spectrometer coupled to a gas chromatograph and showed the three components to be isomers of molecular weight 152. The mass spectrum of peak 2

(1) L. Crombie and M. Elliot, *Fortschr. Chem. Org. Naturstoffe*, **19**, 121 (1961).

(2) M. S. Schechter, N. Green, and F. B. La Forge, *J. Amer. Chem. Soc.*, **71**, 3165 (1949).

(3) B. Tabenkin, R. A. LeMahieu, J. Berger, and R. W. Kierstead, *Appl. Microbiol.*, **17**, 714 (1969).

(4) R. T. Dahill, *J. Org. Chem.*, **31**, 2694 (1966).

(5) Obtained from Benzol Products, Newark, N. J.

(6) The ir spectra were determined using a Beckman IR-9 spectrophotometer. The uv spectra were obtained with a Cary 14 spectrophotometer. The nmr spectra were determined using a Varian HA-100 spectrometer with a C-1024 time-averaging computer when necessary. The mass spectra were obtained using a Finnigan mass spectrometer coupled to a Perkin-Elmer gas chromatograph.