s), as well as, two triplets at 3.38 (2 H) and 4.99 (2 H) with $J = 6$ cps, a result compatible with the substitution pattern found in palmatine. The spectrum differed significantly only in the methoxy region, showing two peaks at 4.08 (3 H) and 4.27 (3 H). The remaining two oxygens must be present as phenolic groups as methylation with dimethyl sulfate gave palmatine chloride, identical in all respects (infrared, ultraviolet, tlc, and mixture melting point) with an authentic sample.

The arranging of two methoxy groups and two phenolic groups on the protoberberine skeleton having the oxygenation pattern of palmatine gives a total of six possible structures. The position of the phenolic groups was determined by methylation of alkaloid B with 1 equiv of dimethyl sulfate and examination of the monophenolic products formed. This was accomplished by separating the reaction mixture by preparative thin layer chromatography utilizing silica gel G and methanol–ammonium hydroxide–water (8:1:1), removing the colored bands, and crystallizing the material from these bands as the chloride salts. There was isolated in this manner palmatine, alkaloid B (starting material), columbamine (III), and dehydrocorydalmine (IV). Identification of the reaction products was by direct comparison of the ultraviolet and infrared spectra and the mobility in the thin layer chromatographic system with authentic samples. Alkaloid B must therefore have the structure V, a new natural product which we have named stepharanine.

Experimental Section

Melting points were determined on a Kofler hot stage and are uncorrected. Infrared spectra were recorded in KBr windows on a Perkin-Elmer Model 237 spectrophotometer. Ultraviolet spectra were determined in ethanol on a Cary Model 15 spectrophotometer. Proton magnetic resonance spectra were obtained in trifluoroacetic acid with tetramethylsilane as internal standard on a Varian A-60A apparatus. Thin layer chromatography was performed on silica gel G (Merck) plates with methanol–ammonium hydroxide–water (8:1:1) as the solvent with detection by visual examination as the alkaloids are colored.

Methylation of Dehydrocorydalmine (Alkaloid A) to Palmatine.—Dehydrocorydalmine chloride (20 mg) was dissolved in 20 ml of water and 2 ml of a saturated sodium bicarbonate solution was added. The solution immediately turned orange and 4 mg of dimethyl sulfate was added. The reaction mixture was stirred at room temperature for 6 hr and then evaporated to dryness at reduced pressure. The residue was dissolved in 10 ml of hot water and on cooling overnight deposited fine yellow needles which after collecting, washing with cold water, and drying weighed 15 mg, mp 203–205°. When this product was admixed with authentic palmatine chloride, it had mp 203–205° and the infrared spectra of the two were superimposable. Mobility in the thin layer system was R_f 0.12.

Methylation of Stepharanine (Alkaloid B) with Dimethyl Sulfate (1 Equiv).—Stepharanine chloride (50 mg) was dissolved in 40 ml of water and 4 ml of a saturated sodium bicarbonate solution was added. The precipitate that formed was redissolved by warming on the steam bath. Dimethyl sulfate (18 mg) was added and the solution was stirred magnetically for 2 hr at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in 10 ml of absolute ethanol, filtered, and the filtrate reduced to 2 ml to be spotted on thin layer plates. Five silica gel G thin layer plates (20 × 20 cm) were poured to a thickness of 1 mm, then dried and activated in an oven at 150°. The 2-ml reaction mixture solution was streaked evenly over the five plates and developed by methanol–ammonium hydroxide–water (8:1:1). Examination of the developed plates under daylight and ultraviolet light showed four major zones at R_f 0.12, 0.16, 0.60, and 0.67 corresponding to palmatine, columbamine, dehydrocorydalmine, and stepharanine, respectively.

The material from the four zones was isolated in the following manner. After removing the colored bands, the substances were dissolved away from the adsorbent with methanol. The solvent was removed by evaporation to dryness under reduced pressure. The residue was dissolved in 1 ml of hot methanol, filtered, and on cooling the filtrate deposited crystalline material. The zone with R_f 0.12 yielded 6 mg of yellow crystals, mp 202–204°, showing infrared and ultraviolet spectra identical with palmatine chloride. The material (5 mg) from the zone with R_f 0.16 melted at 238–241° and showed infrared and ultraviolet spectra identical with columbamine chloride, mmp 238–240°. The R_f 0.60 zone gave 4 mg of a material, mp 219–221°, identical in infrared and ultraviolet spectra with dehydrocorydalmine, mmp 219–221°. The material from the zone with R_f 0.67 was identical with the starting material, stepharanine.

Registry No.—IV chloride, 13509-85-8; IV iodide, 13509-86-9; V chloride, 13509-87-0.

Isolation, Structure, and Synthesis of Hymenoxin, a New Flavone from *Hymenoxys scaposa* (Compositae)

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An investigation[1] of the infraspecific variation of the flavonoid constituents in the Compositae species, *Hymenoxys scaposa*, has led to the isolation, structure determination, and synthesis of a new member of the rare group of fully oxygenated A-ring flavones.[2] When the A-ring substituents are as shown in **1a** neither proton nuclear magnetic resonance (pmr) nor ultraviolet spectral data will unequivocally locate the positions of all the hydroxyl and methoxyl groups [*cf.* lucidin[3] (5,7-dihydroxy-6,8-dimethoxy-3′,4′-methylenedioxyflavone), nevadensin[4] (5,7-dihydroxy-4′,6,8-trimethoxyflavone), acerosin[5] (3′,5,7-trihydroxy-4′,6,8-trimethoxyflavone), and sudachitin[5] (4′,5,7-trihydroxy-3′,6,8-trimethoxyflavone)].

Methylene chloride extraction of the leaves of *Hymenoxys scaposa* (Family Compositae) collected near Austin, Texas, yielded a crystalline flavone, $C_{19}H_{18}O_8$, mp 211–213°, which we named hymenoxin. The flavone nucleus and the oxygenation pattern were suggested to be the same as those of **1** by the ultraviolet and pmr spectra. The pmr spectrum of hymenoxin trimethylsilyl ether[6] indicated the presence of four methoxyl and two hydroxyl groups, the latter as trimethylsilyl signals. Hymenoxin mono(trimethylsilyl ether) exhibited a pmr singlet at 12.53 ppm, a signal typical for a C-5 hydrogen-bonded hydroxyl group. The ultraviolet spectra of hymenoxin in methanol alone and methanol with diagnostic reagents (sodium methoxide,

(1) P. Seeligmann and R. E. Alston, *Brittonia*, **19**, 205 (1967).

(2) J. B. Harborne, "Comparative Biochemistry of the Flavonoids," Academic Press Inc., New York, N. Y., 1967, pp 42–46.

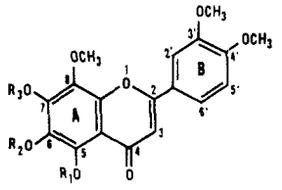
(3) H. H. Lee and C. H. Tan, *J. Chem. Soc.*, 2743 (1965).

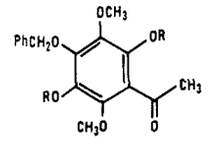
(4) L. Farkas, M. Nogradi, V. Sudarsanam, and W. Herz, *J. Org. Chem.*, **31**, 3228 (1966).

(5) L. Farkas, M. Nogradi, V. Sudarsanam, and W. Herz, *Tetrahedron*, in press.

(6) T. J. Mabry, J. Kagan, and H. Rösler, "Nuclear Magnetic Resonance Analysis of Flavonoids," The University of Texas Publication No. 6418, Austin, Texas, 1964.

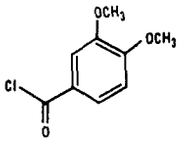
aluminium chloride, and sodium acetate)[7] suggested a close structural relationship with lucidin,[3] nevadensin,[4] sudachitin,[5] and acerosin.[5] Although the latter compounds all have a free 7-hydroxyl group, none produces the expected[7] 8–20-mμ bathochromic shift of band II in the presence of fused sodium acetate. Thus, since it is not always possible to determine from spectral data the location of the methoxyl and hydroxyl groups, a total synthesis of flavones with fully oxygenated A rings is required.





1a, $R_1 = R_3 = H$; $R_2 = CH_3$
b, $R_1 = CH_3$; R_2 = 3,4-dimethoxybenzoyl; R_3 = benzyl
c, $R_1 = CH_3$; $R_2 = H$; R_3 = benzyl
d, $R_1 = CH_3$; $R_2 = CH_3$; R_3 = benzyl

2a, R = H
b, R = 3,4-dimethoxybenzoyl



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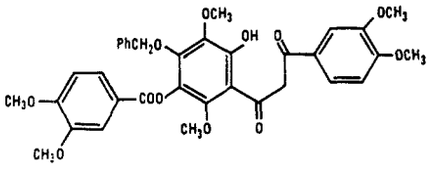

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A total synthesis of hymenoxin was therefore undertaken by the route outlined below.[4,5] 4-Benzyloxy-2,5-dihydroxy-3,6-dimethoxyacetophenone[4] (**2a**) was converted into **2b** on acylation with veratroyl chloride (**3**). The Baker–Venkataraman transformation[8] of **2b** gave the dibenzoyl methane derivative (**4**), which was subsequently cyclized to 7-benzyloxy-6-(3,4-dimethoxybenzoyloxy)-3′,4′,5,8-tetramethoxyflavone (**1b**). Alkaline hydrolysis of **1b** to **1c** followed by methylation with dimethyl sulfate gave the pentamethoxy compound **1d**. The latter substance was treated with aluminium chloride in dry ether producing 5,7-dihydroxy-3′,4′,6,8-tetramethoxyflavone (**1a**), which was identical in all respects with the natural product, hymenoxin. Aluminium chloride in dry ether not only debenzoylated the 7 position but also selectively demethylated the 5 position.

Experimental Section[9]

Isolation of Hymenoxin.—Dried, ground leaves of *Hymenoxys scaposa*[10] (196 g) collected approximately 25 miles northwest of Austin, Texas, April 23, 1966, were extracted with cold petroleum ether (2 l., 48 hr) and then with cold dichloromethane (three 1-l. portions, 48 hr). The combined dichloromethane extracts yielded 13 g of crude gum which was taken up in chloroform and chromatographed over silica gel. The first fractions, which were eluted with chloroform–methanol (99.5:0.5), solidified on treatment with carbon tetrachloride and weighed 110 mg. The residue was rechromatographed and the material thus obtained was recrystallized three times from benzene to yield 20 mg of yellow prisms: mp 211–213°; ultraviolet, λ_{max} 279 mμ (ε 21,400) and 337 mμ (ε 22,000); λ_{max} (with sodium methoxide) 281, 308 (infl), and 376 mμ; λ_{max} (with $AlCl_3$ and HCl) 257, 293, and 362 mμ; λ_{max} (with sodium acetate) 281, 318 (infl), and 374 mμ; infrared bands (KBr), 1655 (ketone), 1620, 1585, and 1518 (aromatics) cm^{-1}; pmr of trimethylsilyl ether (CCl_4), 7.35–7.63 m (H-2′ and H-6′), 6.87 d (J = 8 cps, H-5′), 6.35 (H-3), 3.90, 3.86, 3.86, and 3.73 (four methoxyl singlets), and 0.27 and 0.30 ppm (two trimethylsilyl ether singlets). Hymenoxin mono(trimethylsilyl ether) had pmr signals at 12.53 (C-5 hydroxyl group) and 6.46 ppm (H-3).

Anal. Calcd for $C_{19}H_{18}O_8$: C, 60.95; H, 4.8. Found: C, 61.0; H, 5.0.

4-Benzyloxy-3,6-dimethoxy-2,5-di(3,4-dimethoxybenzoyloxy)-acetophenone (2b).—A mixture of 4-benzyloxy-2,5-dihydroxy-3,6-dimethoxyacetophenone[4] (**2a**, 0.6 g) and veratroyl chloride (**3**, 1.12 g) in pyridine (3 ml) was heated on a boiling water bath for 30 min and poured into 15 ml of 5% hydrochloric acid solution. Chloroform extraction of the acidic solution yielded a residue which on crystallization from chloroform–methanol afforded colorless needles (941 mg, 77%): mp 176.5–177°; ultraviolet, λ_{max} 264 mμ (ε 35,400) and 295 mμ (ε 23,200); infrared bands (KBr), 1740 (ester carbonyl), 1704 (ketone), and 1602 and 1520 (aromatics) cm^{-1}.

Anal. Calcd for $C_{35}H_{34}O_{12}$: C, 65.0; H, 5.3. Found: C, 65.2; H, 5.4.

7-Benzyloxy-6-(3,4-dimethoxybenzoyloxy)-3′,4′,5,8-tetramethoxyflavone (1b).—A mixture of **2b** (0.6 g) and powdered potassium hydroxide (*ca.* 80 mg) was heated with pyridine (3 ml) at 60° for 3 hr with stirring. After this was mixed with 10 ml of 2.5% hydrochloric acid, the resulting solution was extracted with chloroform. The residue obtained from the chloroform layer was refluxed with 1.5% ethanolic sulfuric acid (6 ml) for 1 hr. The solution was diluted with water and extracted with chloroform. The chloroform extract was washed with sodium bicarbonate solution, dried with anhydrous magnesium sulfate, and evaporated. The residue crystallized from methanol as colorless needles (375 mg), which melted at 166°, solidified, and remelted at 182°. The flavone exhibited ultraviolet peaks at λ_{max} 250 mμ (infl) (ε 27,500), 267 (33,400), 300 (infl) (22,200), and 330 (25,400); infrared bands (KBr) at 1739 (ester carbonyl), 1647 (ketone), and 1604, 1573, and 1520 (aromatics) cm^{-1}; and a characteristic pmr signal at 6.64 ppm (H-3).

Anal. Calcd for $C_{35}H_{32}O_{11}$: C, 66.9; H, 5.1. Found: C, 66.7; H, 5.3.

7-Benzyloxy-6-hydroxy-3′,4′,5,8-tetramethoxyflavone (1c).—Compound **1b** (200 mg) was refluxed in 1 *N* sodium methoxide (1.2 ml) for 1 hr. The reaction mixture was acidified with acetic acid and diluted with water to precipitate the flavone **1c** (109 mg, 74%). Crystallization of the material from methanol afforded colorless prisms: mp 181°; ultraviolet, λ_{max} 278 mμ (ε 20,500) and 332 mμ (ε 25,700); infrared bands (KBr), 1635 (ketone), and 1603, 1577, 1518 (aromatics) cm^{-1}; pmr, 6.62 (H-3), 5.33 (benzyl methylene protons), and 3.93 and 3.97 ppm (four methoxyls).

Anal. Calcd for $C_{26}H_{24}O_8$: C, 67.25; H, 5.2. Found: C, 67.4; H, 5.3.

7-Benzyloxy-3′,4′,5,6,8-pentamethoxyflavone (1d).—Compound **1c** (100 mg), dimethyl sulfate (0.6 ml), and anhydrous potassium carbonate (0.6 g) were refluxed in acetone (15 ml) for 4 hr. The inorganic salts were filtered and the solvent was removed. The residue was crystallized from methanol as long colorless needles (52 mg): mp 160°; ultraviolet, λ_{max} 246 mμ (ε 22,100), 267 (20,400), and 330 (26,500); infrared bands (KBr), 1645 (ketone), and 1602, 1590, 1568, and 1518 (aromatics) cm^{-1}; pmr, 6.59 (H-3), 5.28 (benzyl methylene protons), and 3.90, 3.95, and 3.97 ppm (five methoxyl signals).

Anal. Calcd for $C_{27}H_{26}O_8$: C, 67.8; H, 5.4. Found: C, 68.3; H, 5.7.

5,7-Dihydroxy-3′,4′,6,8-tetramethoxyflavone (Hymenoxin, 1a).—Compound **1d** (30 mg) was added to a solution of anhydrous aluminum chloride (0.52 g) in ether (2.6 ml) and allowed to stand at room temperature for 3 hr. The solvent was removed and the residue was treated with 6.5 ml of ice-cold, 18% aqueous

(7) L. Jurd, "Spectral Properties of Flavonoid Compounds," in "The Chemistry of Flavonoid Compounds," T. A. Geissman, Ed., The Macmillan Co., New York, N. Y., 1962, pp 107–155.

(8) J. Gripenberg, "Flavones," in "The Chemistry of Flavonoid Compounds," pp 410–411.

(9) Melting points are uncorrected. Ultraviolet spectra were determined in absolute methanol. Unless otherwise stated, pmr spectra were determined in deuteriochloroform with tetramethylsilane as internal reference and are reported with the following abbreviations: s = singlet, d = doublet, and m = complex multiplet; coupling constants (J) are given in cycles per second (cps). Analyses were determined by Dr. Alfred Bernhardt, Max-Planck Institut für Kohlenforschung, Mülheim, West Germany.

(10) Voucher No. 255332, The University of Texas Herbarium, Austin.

hydrochloric acid. The reaction mixture was warmed on a steam bath for 5 min, cooled, and extracted with chloroform. Crystallization (from chloroform–carbon tetrachloride) of the residue from the chloroform extract afforded 17 mg (72%) of crude flavone. The material was chromatographed over silica gel using chloroform as eluent and finally recrystallized from benzene: yield, 5 mg of yellow prisms; mp and mmp 211–213° with natural hymenoxin. The infrared, ultraviolet, and pmr spectra of the synthetic material were identical with those observed for hymenoxin isolated from *Hymenoxys scaposa*.

Registry No.—**1a**, 13509-93-8; **1b**, 13509-94-9; **1c**, 13509-95-0; **1d**, 13509-96-1; **2b**, 13509-97-2.

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Synthesis of N^{α}-Benzoyl-S-2-aminoethyl-L-cysteine Amide Hydrobromide[1]

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In connection with studies on the relative rates of tryptic hydrolysis of arginyl, lysyl, and S-2-aminoethyl-L-cysteinyl (AEC) peptide bonds, the amide of N^{α}-benzoyl-S-2-aminoethyl-L-cysteine (**7**) was needed. This paper describes the synthesis of this compound.

The procedure was modeled on Hofmann and Bergmann's synthesis of N^{α}-benzoyllysinamide[2] (Scheme I). Cysteine hydrochloride was allowed to react with ethylenimine in aqueous solution to give AEC (**1**) as the hydrochloride. This synthesis of AEC, which is based on the aminoethylation procedure of Raftery and Cole,[3] is superior to previous syntheses of this compound[3-5] in that the AEC is obtained directly in good yield and is free of contaminating salts. The AEC was acylated with benzyloxycarbonyl chloride to give di-(benzyloxycarbonyl)-AEC (**2**) which in turn was converted to the Leuchs' anhydride **3** by treatment with phosphorous pentachloride. Although this anhydride could be isolated in crystalline form, it was convenient to convert it directly to the ester **4b** by treatment with methanol. Following benzoylation of the α-amino group, the ester group in **5b** was ammonolyzed to give the amide **6**. The benzyloxycarbonyl group in the latter compound was removed by hydrogen bromide in acetic acid[6] to yield the desired N^{α}-benzoyl-AEC amide (**7**) as its hydrobromide salt. All of the reactions proceeded smoothly in good yield (53–93%), and the final product was readily obtained in crystalline form. The procedure also gave ready access to N^{α}-benzoyl-AEC ethyl and methyl esters (**8**), which are also substrates for trypsin.

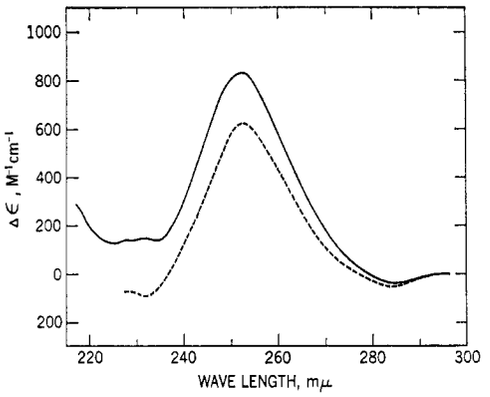

Figure 1.—Difference spectra of N^{α}-benzoyl-AEC-amide *vs.* N^{α}-benzoyl-AEC (- - - -) and N_{α}-benzoyl-AEC ethyl ether *vs.* N^{α}-benzoyl-AEC (———), both in pH 9.0, 0.05 *M* sodium borate buffer.

The reaction of benzaldehyde with lysine gives the N^{ϵ}-benzylidine derivative which is useful for the preparation of α-substituted compounds of lysine.[7] AEC behaves in a manner similar to lysine in that the benzaldehyde reacted exclusively with the ω-amino group. The resulting N^{ω}-benzylidine derivative **9** was treated with benzoyl chloride in alkali to yield, after acidification, N^{α}-benzoyl-AEC (**10**). The latter compound was identical with that prepared by the action of trypsin on N^{α}-benzoyl-AEC methyl ester (**8b**).

The amide **7**, ester **8**, and free acid **10** all exhibit an absorption maximum at 228 mμ with a molar extinction coefficient of 1.14–1.19 $\times$ 10^4 M^{-1} cm^{-1}. However, the free acid has an enhanced absorption at 250–260 mμ which leads to a marked difference spectra between the acid and the amide or the ester in this region. The difference spectra exhibit a peak at 253 mμ having a $\Delta\epsilon$ of 620 and 830 M^{-1} cm^{-1} for the amide and ester, respectively (Figure 1). Advantage can be taken of this difference in absorption to follow the rate of tryptic cleavage of the amide or ester. It should be noted that the corresponding N^{α}-benzoyl derivatives of glycine, lysine, and arginine exhibit similar difference spectra.

Experimental Section[8]

S-2-Aminoethyl-L-cysteine Hydrochloride (**1**).—L-Cysteine hydrochloride (15.7 g, 0.1 mole) was dissolved in 70 ml of water and the solution was cooled in an ice bath. Ethylenimine (5.7 ml, 0.115 mole) was added with stirring along with a few drops of 0.1% phenolphthalein. The solution was then titrated to a slight pink color with more ethylenimine ($\backsim$1 ml) and the stirring was continued for 30 min. At the end of this period, the nitroprusside test for the free SH group was very weak. The reaction mixture was evaporated to one-half of the original volume and an equal volume of ethanol was added. After storage of the mixture at 4° overnight, the solid mass of crystals was collected and recrystallized from water with ethanol to yield 15.1 g (75%): mp 194–195° dec, $[\alpha]^{27}D$ −4.2°, $[\alpha]^{27}_{380}$ −7.6, $[\alpha]^{27}_{230}$ +657°

(1) This work was supported in part by Grant AM-00608 from the National Institutes of Health and Grant GB-5931 from the National Science Foundation.

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(3) M. A. Raftery and R. D. Cole, *Biochem. Biophys. Res. Commun.*, **10**, 467 (1963); *J. Biol. Chem.*, **241**, 3457 (1966).

(4) (a) D. B. Hope and K. C. Horncastle, *J. Chem. Soc.*, 1098 (1966); (b) D. Cavallini, C. de Marco, B. Mondovi, and G. F. Azzone, *Experimentia*, **11**, 61 (1955); (c) K. Blaha, I. Fric, and P. Hermann, *Collection Czech. Chem. Commun.*, **30**, 304 (1965).

(5) H. Lindley, *Nature*, **178**, 647 (1956); *Australian J. Chem.*, **12**, 296 (1959).

(6) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

(7) B. Bezas and L. Zervas, *J. Am. Chem. Soc.*, **83**, 719 (1961).

(8) The ultraviolet spectra and the difference spectra were taken on a Cary Model 15 spectrophotometer. The specific rotations were measured on a Cary Model 60 spectropolarimeter. Unless otherwise stated, a Radiometer TTT1c pH-Stat was used whenever the maintenance of the pH of a reaction was required. The elementary analyses were performed by the Chemistry Department, University of California, Berkeley, Calif. Melting points were determined in open capillaries in a Hershberg apparatus and are uncorrected.