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CONSTITUTION OF VERBENALIN[1]

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Abstract—In agreement with earlier work catalytic reduction of verbenalin gave tetrahydroverbenalin and a hydroxy-δ-lactone, desoxyverbanol. Oxidation of desoxyverbanol furnished a keto lactone which was converted to (+)-iridomyrmecin of known relative and absolute configuration by desulfurization of its ethylenethioketal. The formation of β-methylglutaric acid on ozonation of verbenalin allows placement of the ketone function in the glucoside. Verbenalin was shown to be a methyl ester by hydrolysis to the corresponding acid which could be reconverted to the natural product by esterification with diazomethane. Comparison of the UV spectra of verbenalin and verbenalol with those of tetrahydro-desoxyplumieride and bakankosin derivatives revealed the nature of the chromophore. Configurations are assigned to all asymmetric centers and a possible relationship of verbenalin to loganin is discussed.

THE glycoside verbenalin, $C_{17}H_{24}O_{10}$, first isolated from *Verbena officinalis*[3] and later from *Verbena stricta*,[4] has been shown to be identical with cornin[5] from *Cornus florida* L.

Early investigators showed it to be a β-D-glucoside, and further, established the presence of one carbon-carbon double bond[6] and a keto group.[6,7] Kuhn-Roth oxidation indicated the presence of one C-methyl group, while a Zeisel determination detected one methoxyl function. The ultraviolet spectrum of verbenalin, λ_{max}^{EtOH} 238 mμ (ε 9,600) was previously ascribed[8] to an α,β-unsaturated ketone function and infrared absorption (in KBr) at 1730, 1685, and 1640 cm^{-1} was attributed to two carbonyl groups and a double bond. There was disagreement among early workers regarding the nature of the second carbonyl group. One group[9] considered verbenalin to be a methyl ester, while others[7,8] postulated a lactone function.

Verbenalin can be hydrolyzed enzymatically with emulsin to the aglycone, verbenalol, with the expected molecular composition, $C_{11}H_{14}O_5$. The ultraviolet spectrum and the infrared absorptions in the 1600 to 1800 cm^{-1} region of the aglycone are essentially identical with those of the glucoside, and a band at 3500 cm^{-1} was attributed to the newly created hydroxyl function. However, verbenalol, contrary to verbenalin gives positive ferric chloride and Tollens tests, and these findings can only be rationalized if the hydroxyl group imparts enolic character to the aglycone.

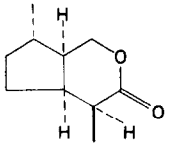
[1] A portion of this study has been reported in a preliminary communication. G. Büchi and R. E Manning, *Tetrahedron Letters* No 26, 5 (1960).
[2] Holder of National Science Foundation Summer Fellowships 1960–1961.
[3] L. Boudier, *J. Pharm. Chim.* **27**, 49, 101 (1908).
[4] B. Reichert, *Arch. Pharm.* **273**, 357 (1935).
[5] A. Chatterjee and L. M. Parks, *J. Amer. Chem. Soc.* **71**, 2249 (1949).
[6] W. Hoffmann, *Arch. Pharm.* **281**, 269 (1943).
[7] J. Cheymol, *Bull. Soc. Chim.* **5**, 633, 642 (1938).
[8] M. Cohn, E. Vis, and P. Karrer, *Helv. Chim. Acta* **37**, 790 (1954); P. Karrer and H. Salomon, *Helv. Chim. Acta* **29**, 1544 (1946).
[9] J. Asano, Y. Ueno and Y. Tamaki, *J. Pharm. Soc. Japan* **62**, 7 (1942).

Catalytic hydrogenation of verbenalin yielded the anticipated tetrahydroverbenalin, and a small amount of a glucose-free compound, desoxyverbanol. Tetrahydroverbenalin exhibited no high-intensity ultraviolet absorption and only one band (1713 cm,$^{-1}$ in KBr) appeared in the 1600 to 1800 cm^{-1} region of its infrared spectrum. Apparently both the carbon-carbon double bond and the ketone function present in the natural product had been reduced. Enzymatic cleavage[8] of tetrahydroverbenalin furnished tetrahydroverbenalol with no ultraviolet maximum above 210 mμ and the anticipated infrared peaks at 1730 and 3580 cm^{-1} (in chloroform). Desoxyverbanol, $C_{10}H_{16}O_3$, a by-product obtained in this hydrogenation contained no methoxyl group[8] (Zeisel determination) and two C-methyl groups (Kuhn-Roth). Its infrared spectrum (in chloroform) had bands at 3550 and 1750 cm^{-1}, attributable to hydroxyl and lactone groups respectively and the presence of the former functionality was confirmed by preparation[8] of a monotosylate.

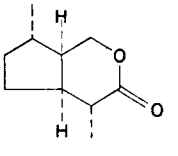
Catalytic reduction of verbenalol under similar conditions did not yield the aforementioned tetrahydroverbenalol as might have been anticipated, but a new compound, norverbanol, $C_{10}H_{16}O_4$ with distinctive infrared absorption (in chloroform) at 3500 and 1750 cm^{-1} which were assigned to hydroxyl and lactone functions respectively. The presence of two hydroxyl groups was ascertained by formation[8] of dibenzoyl and di-*p*-nitrobenzoyl derivatives. Comparison of the molecular compositions of desoxyverbanol and norverbanol suggests that the latter is simply a hydroxy derivative of the former.

As previously mentioned, desoxyverbanol contains one hydroxyl group, one lactone function and two C-methyl groups, and hence must be bicyclic. The frequency of the carbonyl band (1750 cm^{-1}) in the infrared spectrum is suggestive of a δ-lactone and oxidation yielded a ketone, desoxyverbanone, $C_{10}H_{14}O_3$, with no hydroxyl absorption in the infrared region and only one carbonyl band at 1745 cm^{-1}, arising from the superposition of lactone and ketone peaks. It follows that desoxyverbanone contains a δ-lactone and a cyclopentanone system.

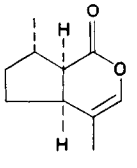
The functionalities now known to be present in desoxyverbanone are also found in the naturally-occurring monoterpenoid lactones iridomyrmecin,[10] I, isoiridomyrmecin,[10] II and nepetalactone[11] III, and we made the biogenetically reasonable



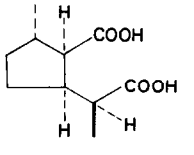
I



II



III



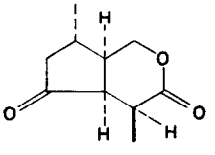
IV

[10] G. W. K. Cavill and D. L. Ford, *Austr. J. Chem.* **13**, 296 (1960) and references given there.

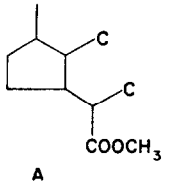
[11] R. B. Bates, E. J. Eisenbraun and S. M. McElvain, *J. Amer. Chem. Soc.* **80**, 3420 (1958) and earlier papers cited.

assumption that desoxyverbanone possesses the same isoprenoid carbon skeleton. To confirm this theory we had to correlate desoxyverbanone with one of the three natural products or with one of the isomeric nepetalinic acids.[11] Such a transformation was realized when desulfurization of desoxyverbanone ethylenethioketal with Raney-nickel yielded (+)-iridomyrmecin,[10,12] I. Moreover, oxidation of I with potassium permanganate produced a dicarboxylic acid which was identical in every respect with the nepetalinic acid[11,13] IV. Placement of the carbonyl group in desoxyverbanone (V) is consonant with the formation of β-methylglutaric acid upon ozonization of tetraacetylverbenalin.

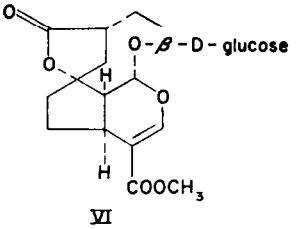
With the structure of desoxyverbanone established, we can now turn to a discussion of its relationship to verbenalin which became clear only after the controversial nature of the carboxyl group had been elucidated. Hydrolysis of verbenalin with barium hydroxide generated verbenalinic acid,[7] $C_{16}H_{22}O_{10}$, which was reconverted to verbenalin when treated with diazomethane. These transformations left no doubt that verbenalin is a methyl ester, and the lactone group in desoxyverbanol must arise from the carbomethoxy function in verbenalin, which can now be represented by the partial formula A.



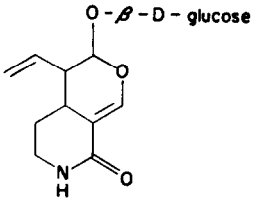
V



A



VI

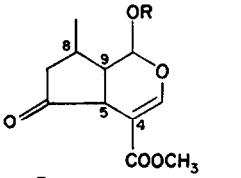


VII

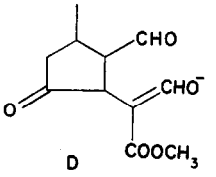
The structure of the chromopore in verbenalin could be inferred from a comparison of its ultraviolet spectrum, λ_{max}^{EtOH} 238 mμ (ϵ 9,600) with those of tetrahydrodesoxyplumieride,[14] VI, λ_{max}^{EtOH} 236 mμ (ϵ 10,000) and bakankosin,[1,15] VII, λ_{max}^{EtOH} 236 mμ (ϵ 11,600). Consequently verbenalin was assigned structure B and in agreement with this view, verbenalol (C) λ_{max}^{EtOH} 240 mμ (ϵ 9,050) had λ_{max}^{EtOH} 271 mμ (ϵ 19,000) in 0·01 N sodium hydroxide solution. The bathochromic displacement can be attributed to production of the enolate anion D and the magnitude of the

[12] We are indebted to Dr. G. W. K. Cavill for this comparison.
[13] Professors H. L. Goering and S. M. McElvain, private communication.
[14] O. Halpern and H. Schmid, *Helv. Chim. Acta* **41**, 1109 (1958).
[15] G. Büchi, unpublished. K. Balenovic, H. U. Däniker, R. Goutarel, M. M. Janot and V. Prelog, *Helv. Chim. Acta* **35**, 2519 (1952).

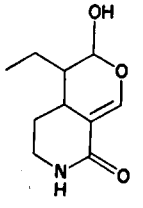
effect ($\Delta\lambda$ 31 mμ) corresponds closely to that for ethyl acetoacetate ($\Delta\lambda$ 33 mμ)[16] and dihydrobakankogenin,[1,15] VIII, ($\Delta\lambda$ 38 mμ). Norverbanol, IX, has only one C-methyl group and consequently one of the two hydroxyl functions must be located on the methyl group α to the lactone carbonyl.



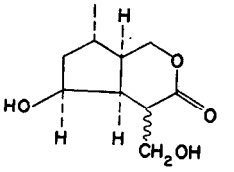
B, R = β-D-glucose
C, R = H



D



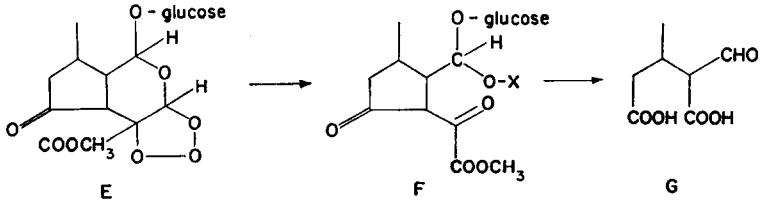
VIII



IX

Before turning to the stereochemistry of verbenalin we must discuss its oxidation with three molar equivalents of ozone followed by further degradation with hydrogen peroxide. The formation of β-methylglutaric, methylsuccinic and oxalic acids can be rationalized if it is assumed that an initially formed molozonide (E) suffers decomposition to the keto ester (F) either directly or via an ozonide. Both enol forms of F should be susceptible to further attack by ozone yielding intermediates from which the oxalyl moiety can be lost by fragmentation. The remaining portion of the molecule (G) can either lose formic acid or undergo further oxidation to methylsuccinic acid.

The stereochemistry of the four asymmetric centers (C_1, C_5, C_8 and C_9) present in the aglycone portion of verbenalin was ascertained as follows. (+)-Iridomyrmecin seems to have the relative and absolute configuration shown in I,[17–20] and desoxyverbanone consequently must be represented by V.

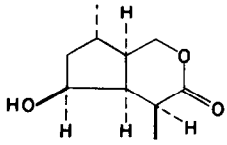


E F G

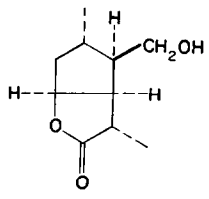
Because reduction of desoxyverbanone with sodium borohydride regenerated desoxyverbanol, no epimerization has occurred in the oxidation, and the latter can

[16] P. Grossmann, *Z. physik. Chem.* **109**, 305 (1924).
[17] R. H. Jaeger and Sir Robert Robinson, *Tetrahedron Letters* No 15, 14 (1959).
[18] L. Dolejs, A. Mironov and F. Sorm, *Tetrahedron Letters* No 11, 18 (1960). E. J. Eisenbraun, T. George, B. Riniker and C. Djerassi, *J. Amer. Chem. Soc.* **82**, 3648 (1960).
[19] G. W. K. Cavill, personal communication.
[20] F. Korte, K. H. Büchel and A. Zschocke, *Chem. Ber.* **94**, 1952 (1961).

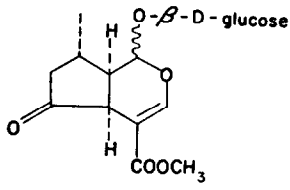
be represented by X if we assume catalyst and hydride approach from the convex side of the molecule. Treatment of desoxyverbanol with excess base followed by acidification results in regeneration of X. Although γ-lactones are usually more stable than δ-lactones angle strain present in XI seemingly reverts this order of stability. The center at C_8 is at no time epimerizable, and thus must have identical configuration in both verbenalin and in iridomyrmecin (I). If we assume for the moment that no epimerization occurred at C_5 or C_9 in the course of catalytic reduction, the natural product must have the geometry pictured in the expanded structure H.



X



XI

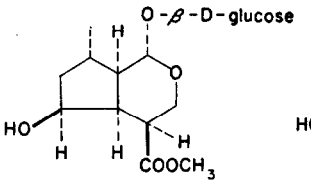


H

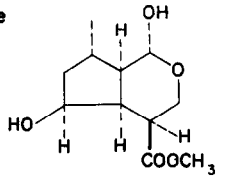
We considered the possibility that verbenalin has the presumably less stable *trans* ring fusion and that isomerization to the *cis* system known to be present in desoxyverbanol had occurred during the catalytic hydrogenation. To test this hypothesis verbenalin was treated with one equivalent of barium hydroxide in deuterium oxide solution, the verbenalinic acid formed isolated and subsequently reesterified with diazomethane. Recrystallization from methanol caused exchange of the deuterium atoms in the four hydroxyl groups and the resulting product contained 2·37 atoms of deuterium, demonstrating that exchange but not epimerization had indeed occurred at C_5. It follows that the angular carbon atoms in verbenalin have the configurations shown in H. Although the exceedingly unlikely possibility that inversion at *both* C_5 and C_9 occurred in the course of the reduction cannot be excluded rigorously, further evidence for a *cis* ring fusion in the glucoside and also for the location of the carbonyl function is provided by the high-intensity n-π^* transition, λ_{max}^{EtOH} 290 mμ (ϵ 105), typical of non-planar β,γ-unsaturated ketones.[21]

It remained to ascertain the configuration at the asymmetric center, C_1, which can be deduced tentatively from a comparison of the optical rotations of verbenalin and plumieride derivatives. The molecular rotation difference $[M]_D$ (tetrahydroverbenalin, XII)—$[M]_D$ (tetrahydroverbenalol, XIII) is −448° (in water) and that for $[M]_D$ (hexahydrodesoxyplumieride, XIV)—$[M]_D$ (corresponding aglycone methyl ether, XV) is −325° (in methanol).[14] The difference in molecular rotation in both cases is of the same sign and of similar magnitude, suggesting tentatively identical configuration at C_1 in both series and the complete constitutions of verbenalin and verbenalol are depicted in XVI and XVII respectively.

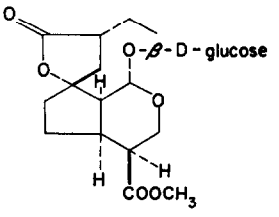
[21] S. F. Mason, *Quart. Rev.* **15**, 287 (1961). (review article).



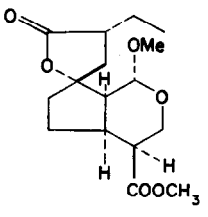
XII



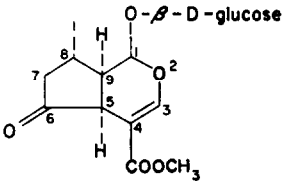
XIII



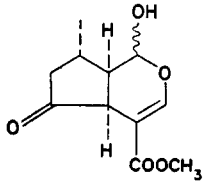
XIV



XV

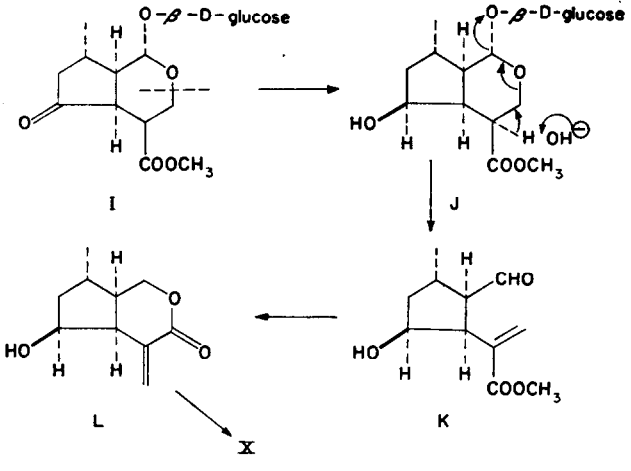


XVI

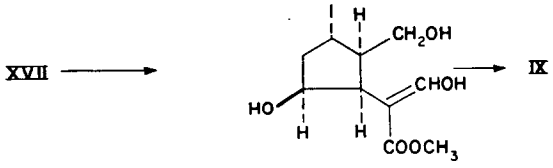


XVII

The formation of desoxyverbanol (X) as a by-product on catalytic reduction of verbenalin deserves comment, particularly because the methyl group α to the lactone carbonyl has the less stable *endo* configuration. This transformation can be envisaged as occurring by hydrogenolysis of the enol oxygen bond (see dotted line in I) to give the intermediate K which can be obtained alternatively by a β-elimination of the oxygen function in tetrahydroverbenalin (arrows in J). Reduction of K followed by lactonization should yield L which on subsequent hydrogenation from the convex side of the molecule is transformed to desoxyverbanol (X).

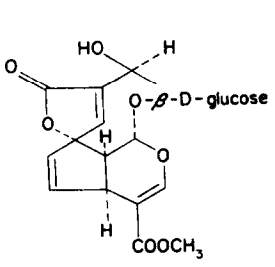


The catalytic hydrogenation of verbenalol (XVII) to norverbanol (IX) can be rationalized by assuming reduction in the open form to give the hypothetical intermediate M. The sequence is terminated by lactonization and further reduction to IX.

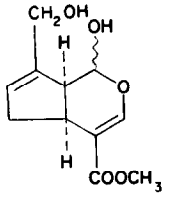


M

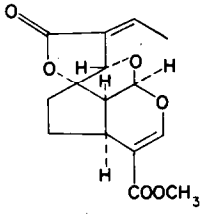
Verbenalin apparently is biogenetically related to plumieride,[14] XVIII, genipin,[22] XIX, plumericin,[23] XX and aucubin,[24] XXI.



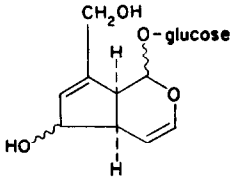
XVIII



XIX



XX



XXI

Loganin ($C_{17}H_{26}O_{10}$) is a glucoside which has been isolated from *Strychnos nux-vomica*[25,26] and from *Strychnos lucida*.[27,28] The structural information available to us[25–28] did not seem to exclude structures XXII and XXIII for this natural product and we decided to synthesize the two epimeric pentaacetates XXIV and XXV. Treatment of verbenalin (XVI) with sodium borohydride yielded a crude pentol which was transformed to XXIV by acetylation. Similarly, tetraacetylverbenalin was

[22] C. Djerassi, T. Nakano, A. N. James, L. H. Zalkow, E. J. Eisenbraun and J. N. Shoolery, *J. Org. Chem.* **26,** 1192 (1961).

[23] G. Albers-Schönberg and H. Schmid, *Helv. Chim. Acta* **44,** 1447 (1961).

[24] W. Haegele, F. Kaplan and H. Schmid, *Tetrahedron Letters*, No. 3, 110 (1961); S. Fujise, H. Obara and H. Uda, *Chem. & Ind.* 289 (1960); J. Grimshaw and H. R. Juneja, *Ibid* 656 (1960); M. W. Wendt, W. Haegele, E. Simonitsch and H. Schmid, *Helv. Chim. Acta* **43,** 1440 (1960).

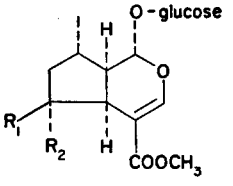
[25] K. W. Merz and K. G. Krebs, *Arch. Pharm.* **275,** 217 (1937).

[26] K. W. Merz and H. Lehmann, *Arch. Pharm.* **290,** 543 (1957).

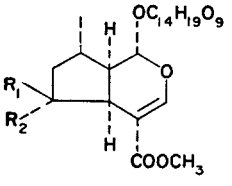
[27] A. J. Birch and E. Smith, *Austr. J. Chem.* **9,** 234 (1956).

[28] A. J. Birch and J. Grimshaw, *J. Chem. Soc.* 1407 (1961).

reduced with the same reagent and the resulting pentoltetraacetate transformed to the monotosylate. Conversion to the desired XXV was accomplished by warming the tosylate with tetraethylammonium acetate in acetone solution. Both XXIV and XXV were different from loganinpentaacetate[29] (mixed m.p. and comparison of infrared spectra). Assuming that loganin and verbenalin do indeed have identical carbon skeletons and configurations the former should be a 7-hydroxy-6-desoxoverbenalin. Further experiments with loganin carried out in another laboratory are consistent with these structures but do not prove either rigorously.[30]



XXII R_1 = OH; R_2 = H
XXIII R_1 = H; R_2 = OH



XXIV R_1 = OAc; R_2 = H
XXV R_1 = H; R_2 = OAc

EXPERIMENTAL

M.ps. were taken on a Kofler Micro Hot Stage and are corrected. IR spectra were recorded on a Perkin-Elmer Model 21 Recording Infrared Spectrophotometer or a Perkin-Elmer Model 137 Infracord. Rotations were determined on a Zeiss polarimeter. The activity of alumina used for chromatographs was determined by the method of Brockmann. Microanalyses were carried out by Mr. W. Egger, Scandinavian Microanalytical Service, Herlev, Denmark.

Verbenalin (XVI) isolated from *Verbena off.* had m.p. 182·2–182·8° $[\alpha]_D$ −173°(*c*, 3·98 water) [Lit[8] m.p. 180–181°, $[\alpha]_D$ −184° (water)]. IR spectrum (KBr): 3400, 2880, 1685, 1640, 1445, 1407, 1360, 1295, 1253, 1222, 1202, 1173, 1135, 1100, 1070, 1030, 993, 955, 927, 912, 889, 873, 856, 818, 797, 772, 690 cm^{-1} UV spectrum: λ_{max}^{EtOH} 238 and 290 mμ (ε 9,600 and 105).

Tetraacetylverbenalin. Verbenalin (2 g) was dissolved in pyridine (20 ml) and acetic anhydride (3·2 ml) was added. After standing overnight at room temp, the brown solution was added dropwise with stirring to a cold, 5% solution of sulfuric acid in water (150 ml). The resultant solid was crystallized several times from 70% ethanol to yield 1·33 g tetraacetylverbenalin, m.p. 133·3–133·8°. [Lit[8] m.p. 133°]. IR spectrum (chloroform): 2880, 1750, 1710, 1640, 1440, 1370, 1230, 1060, 998, 960, 765 cm^{-1} UV spectrum: λ_{max}^{EtOH} 235 mμ (ε 9,370).

Desoxyverbanol (X). Platinized Raney nickel was prepared by treating an aqueous suspension of Raney nickel (4–5 g) with a water solution of potassium chloroplatinate (0·3 g), followed by several washes with water. Verbenalin (8 g) dissolved in water (125 ml), a trace of sodium carbonate and the above catalyst were shaken under hydrogen (270 p.s.i.) for 10 hr at 100°. The catalyst was removed by filtration and the volume of solution reduced under vacuum to about 50 ml and extracted continuously with ether overnight. The ether extract was washed twice with water, dried ($MgSO_4$), and the solvent removed, leaving an oil (1·5 g) which crystallized partially. The crystals were washed several times with ether to remove oily material and crystallization from ethyl acetate–ether yielded 360 mg desoxyverbanol, m.p. 139–140°. [Lit[8] m.p. 137–138°]. IR spectrum (chloroform): 3550,2880, 1745, 1475, 1450, 1380, 1270, 1220, 1170, 1155, 1105, 1080, 1060, 1020, 986, 947 cm^{-1}. (Found: 11·23 (1·38 C—CH_3); Calc. for 2 C—CH_3: 16·30%).

Tetrahydroverbenalin (XII). The aqueous phase which remained after continuous extraction with ether described above was evaporated. Absolute ethanol was added twice to the residue and the solvent evaporated subsequently. The residue failed to crystallize from ethanol over a period of several days and the solution was therefore evaporated to dryness and the residue dissolved in a small amount of water. Continuous extraction with ethyl acetate gave a semi-crystalline mass and after 2 crystallizations from ethanol fine crystals of tetrahydroverbenalin, m.p. 196·3–197·3°, were

[29] We wish to thank Dr. J. Grimshaw for samples of loganin and its pentaacetate.
[30] K. Sheth, E. Ramstad and J. Wolinsky, *Tetrahedron Letters* 394 (1961).

obtained. [Lit[8] m.p. 195–196°]. IR spectrum (KBr): 3300, 2880, 1713, 1450, 1403, 1365, 1325, 1274 1241, 1200, 1175, 1132, 1106, 1075, 1050, 1025, 990, 953, 895, 783 cm^{-1}.

Desoxyverbanone (V). A solution of chromium trioxide (280 mg) in water (10 drops) and acetic acid (25 ml) was added solwly at 0° to a solution of desoxyverbanol (470 mg) in acetic acid (25 ml). After standing overnight at room temp, the solvent was removed under vacuum and the residue treated with several portions of hot ethyl acetate. The combined extracts were washed with water, dried ($MgSO_4$) and the solvent removed. One recrystallization from ethyl acetate-ether yielded 360 mg desoxyverbanone, m.p. 113–114°, $[\alpha]_D$ +57·1° (*c*, 1·77 chloroform). IR spectrum (chloroform): 2990, 1745, 1475, 1450, 1405, 1380, 1340, 1280, 1245, 1160, 1105, 1065, 1013, 1003, 986, 920 cm^{-1} UV spectrum: λ_{max}^{EtOH} 295 mμ (ε 17). (Found: C, 65·65, H, 7·60; Calc. for $C_{10}H_{14}O_3$: C, 65·91, H, 7·74%).

Desoxyverbanone ethylenethioketal. Desoxyverbanone (210 mg) was dissolved in ethanedithiol (0·2 ml) by warming and boron trifluoride etherate (0·2 ml) was added. The solution was kept warm for 30 min and after cooling in an ice bath, ice-cold methanol was added, producing a crystalline precipitate. After washing once with methanol, 260 mg material remained. One recrystallization from methanol yielded 233 mg desoxyverbanone ethylenethioketal, m.p. 191–192°. IR spectrum (chloroform): 3000, 1740, 1440, 1380, 1360, 1285, 1265, 1165, 1120, 1110, 1080, 1020, 990, 975, 950, 880, 850 cm^{-1}.

Iridomyrmecin (I) *from desoxyverbanone ethylenethioketal.* Desoxyverbanone ethylenethioketal (233 mg) was dissolved in absolute ethanol (25 ml), Raney nickel (W-2, 2-3 g) was added and the mixture heated under reflux overnight. The catalyst was collected on a filter and subsequently washed with ethanol and chloroform. Solvent removal under vacuum yielded a yellow amorphous solid (148 mg), which was dissolved in ether and water. The ether phase was washed with water, dried ($MgSO_4$) and the solvent removed, leaving 130 mg crude, crystalline material. Recrystallization from hexane yielded 85 mg iridomyrmecin, m.p. 60·5–61·5° pure and mixed with authentic iridomyrmecin.[12] The IR spectrum (Nujol) was identical with that of iridomyrmecin.[17] $[\alpha]_D$ +236° (*c*, 2·27 chloroform) [Lit[17] $[\alpha]_D$ +210°(CCl_4)].

Nepetalinic acid (IV) *from iridomyrmecin* (I). Iridomyrmecin (57 mg) was dissolved by heating in 3% potassium hydroxide solution (2·5 ml), and after cooling, a 5% solution of potassium permanganate in water (3 ml) was added. After 2 days at room temp, the precipitated manganese dioxide was removed by filtration washed with water and the combined filtrates were acidified with hydrochloric acid to pH 1. The acidic solution was treated with a sufficient amount of sodium bisulfite solution to destroy the excess potassium permanganate. Extraction with ether, drying (Na_2SO_4) and evaporation yielded a colorless oil (73 mg) which was crystallized twice from hexane to give the nepetalinic acid IV, m.p. 115·5–117·5° pure and mixed with an authentic sample.[18] $[\alpha]_D$ +19·5° (*c*, 2·05 chloroform). [Lit[11] $[\alpha]_D$ +19·1°].

Treatment of desoxyverbanol (X) *with base.* Desoxyverbanol (10 mg) was dissolved in water (1 ml) and 1N KOH (1 ml) was added. After 22 hr at room temp, the solution was acidified and extracted with methylene chloride. The combined extracts were washed with water, dried ($MgSO_4$) and the solvent removed to yield 10 mg crystals with an IR spectrum indistinguishable from that of authentic desoxyverbanol.

Sodium borohydride reduction of desoxyverbanone (V). Desoxyverbanone (55 mg) in methanol was added to a methanol–water solution of sodium borohydride. After 4 hr, acetone was added and the solution extracted with 3 portions of methylene chloride. The combined extracts were dried ($MgSO_4$), concentrated and one recrystallization yielded 17 mg desoxyverbanol, m.p. 139–140° pure and mixed with an authentic sample.

Verbenalol (XVII). Pure emulsin (Worthington Biochemical Corp., 200 mg) was dissolved in a citrate buffer solution (40 ml, pH4·6) and water (50 ml). Verbenalin (4·0 g) in water (130 ml) was added and after 24 hr, the solution was extracted continuously with ether overnight. The extract was washed with water, dried ($MgSO_4$) and the solvent removed under vacuum, leaving an oil which crystallized from ether to yield crude verbenalol (1·76 g). Recrystallization from ethyl acetate yielded pure verbenalol, m.p. 125–128° (dec) [Lit[8] m.p. 124°].

IR spectrum (chloroform): 3550, 3000, 1750, 1710, 1640, 1440, 1385, 1305, 1265, 1205, 1190, 1155, 1125, 1103, 1065, 988, 970, 957, 935, 910, 857 cm^{-1} UV spectrum: λ_{max}^{EtOH} 240 mμ (ε 9,050), $\lambda_{max}^{0.01NNaOH}$ 271 mμ (ε 19,000). (Found: 5·07 (0·76 C—CH_3 group) Calc. for 1 C—CH_3: 6·64%).

Norverbanol (IX). Verbenalol (XVII; 1·5 g) in absolute ethanol (125 ml) and Raney nickel

(W-2, 3 g) were shaken at 75° under hydrogen (15 atm) for 8 hr. The catalyst was removed by filtration and the solvent evaporated under vacuum to yield an oil (1·425 g) which crystallized from ethyl acetate to yield 785 mg crude norverbanol. Recrystallization from ethyl acetate afforded 630 mg pure norverbanol, m.p. 97–98·5°. [Lit[8] m.p. 95–96°]. IR spectrum (chloroform): 3500, 3000, 1750, 1455, 1365, 1305, 1185, 1075, 1033, 993, 952, 918, 840 cm^{-1}. (Found: 4·22 (0·56 C—CH_3 group); Calc. for 1 C—CH_3: 7·50%).

Norverbanol monotosylate. Norverbanol (1·00 g, 5 mmoles) was added to a solution of *p*-toluenesulfonyl chloride (1·03 g, 5·4 mmoles) in pyridine (5 ml) and after standing overnight pyridine was removed under vacuum at 45°. Addition of dil hydrochloric acid caused the appearance of a white oil. The mixture was extracted several times with methylene chloride and the combined extracts were washed with a small amount of water and then briefly with potassium bicarbonate solution. The organic phase was dried ($MgSO_4$) and the solvent removed, leaving a colorless oil (1 g) which crystallized on standing. The crystals were triturated with ether, cooled and collected (288 mg). Recrystallization from ethyl acetate-ether furnished pure norverbanol monotosylate, m.p. 127·5–128·5° IR spectrum (chloroform): 3600, 2980, 1750, 1600, 1460, 1360, 1300, 1205, 1190, 1170, 1120, 1095 1070, 1025, 990, 965, 930, 850 cm^{-1}. (Found: C, 57·84, H, 6·25; Calc. for $C_{17}H_{22}O_6S$: C, 57·65, H, 6·26%).

Verbenalinic acid. Barium hydroxide octahydrate (0·407 g, 2·58 mequiv) in water was treated with an aqueous solution of verbenalin (1·00 g, 2·58 mequiv) for 24 hr, after which time the pH was 6. Sulfuric acid solution (2·58 mequiv) was added and the precipitated barium sulfate removed by centrifugation. The aqueous solution was evaporated under vacuum to yield a yellow oil which was dissolved in methanol and evaporated. The remaining oil crystallized upon trituration with ethyl acetate to yield 900 mg crude acid. One recrystallization from methanol–ethyl acetate yielded the product, (700 mg), m.p. 212–214°. [Lit[7] m.p. 210–212°]. IR spectrum (KBr): 1735, 1710, 1630, 1410, 1350, 1257, 1200, 1075, 1043, 1000, 950, 925, 850, 700 cm^{-1}. UV spectrum: λ_{max}^{EtOH} 235 and 300 mμ (ε 6,000 and 130 resp) (Found: C, 51·52, H, 5·97; Calc. for $C_{16}H_{22}O_{10}$: C, 51·33, H, 5·92%).

Verbenalin from verbenalinic acid. Verbenalinic acid (37 mg) dissolved in methanol, was treated with an ether solution of diazomethane until the yellow color persisted after 30 min. Acetic acid in methanol was added and the solution evaporated. Trituration of the residue with ethyl acetate yielded crystals (25 mg), m.p. 162–166°. Two recrystallizations from methanol–ethyl acetate yielded verbenalin, m.p. 176–177·5°, mixed m.p. 177–179°. An IR spectrum was identical with that of authentic verbenalin.

Deuterated verbenalinic acid. Verbenalin (1·00 g, 2·58 mmoles) was added to a solution of barium hydroxide octahydrate (407 mg, 2·58 mequiv) in deuterium oxide. After standing for about 24 hr, sulfuric acid (2·58 mequiv) in deuterium oxide was added, precipitated barium sulfate removed by centrifugation and the aqueous solution evaporated *in vacuo*. The resulting foam was taken up in methanol, the solution evaporated to dryness and the amorphous residue digested with ethyl acetate. After the solvent had been decanted the residue was crystallized twice from methanol–ethyl acetate yielding 600 mg product, m.p. 211–213°.

Deuterated verbenalin from deuterated acid. Deuterated verbenalinic acid (400 mg) in methanol was treated with diazomethane in ether and methylene chloride. After 30 min a few drops of acetic acid were added and the solution stirred with calcium carbonate for 2 hr. The solution was filtered, evaporated and the residue triturated with ethyl acetate to yield 280 mg crude verbenalin. Five recrystallizations from methanol–ethyl acetate yielded 140 mg verbenalin, m.p. 182–183·5°, mixed with verbenalin, m.p. 181–182·5°. (Found: 9·88% (2·37D); Calc. for 3 atoms of deuterium: 12·5%).

Pentaacetyldihydroverbenalin (XXIV). Verbenalin (1·5 g) in absolute methanol (25 ml) was treated with excess sodium borohydride. After standing at room temp for 40 min, acetone and acetic acid were added and the solution evaporated. The resulting oil was dissolved in pyridine (15 ml) and acetic acid anhydride (5 ml). After standing overnight, the solution was slowly added to 120 ml cold 5% aqueous sulfuric acid and cooled. The solid precipitate was collected and washed with water. Two recrystallizations from 75% aqueous ethanol yielded 900 mg product, m.p. 172–174°. Three additional recrystallizations from acetone–hexane yielded an analytical sample.

IR spectrum (KBr): 1735, 1700, 1645, 1440, 1385, 1370, 1295. 1250, 1225, 1175, 1120, 1085, 1065, 1040, 1015, 985, 910, 900, 860, 850, 820, 800, 785, 760, 720, 680 cm^{-1} UV spectrum (ethanol): λ_{max} 232 mμ (ε 11,200). (Found: C, 53·80; H, 5·71; Calc. for $C_{27}H_{36}O_{15}$: C, 53·99; H, 6·05%).

Tetraacetyldihydroverbenalin. Tetraacetylverbenalin (3·6 g) was dissolved in 120 ml 85% aqueous

methanol. Sodium borohydride and carbon dioxide gas were intermittently added to maintain a pH of 7 to 8. After 45 min, acetic acid was added and the solution evaporated to 25 ml under vacuum. The aqueous solution was extracted with 3 portions of methylene chloride, and the combined extracts were dried ($MgSO_4$) and evaporated to yield an oil, which failed to crystallize. Chromatography on alumina (activity III) yielded an oil which again did not crystallize.

Tetraacetyldihydroberbenalin monotosylate. Crude tetraacetyldihydroverbenalin (1·77 g) was dissolved in pyridine (8 ml) and tosyl chloride (5 g) was added. After 36 hr at room temp, water and 5% sulfuric acid were added, and the solution extracted with 2 portions of methylene chloride. The combined extracts were dried ($MgSO_4$) and evaporated to yield an oil which crystallized from ether to yield 950 mg crude crystals. Two recrystallizations from acetone–hexane yielded 840 mg pure product, m.p. 156–8°. (Found: C, 53·63; H, 5·60; Calc. for $C_{32}H_{40}O_{16}S$: C, 53·90; H, 5·66%). IR spectrum (chloroform): 1750, 1705, 1635, 1600, 1435, 1365, 1290, 1230, 1190, 1175, 1095, 1080, 1060, 1040. 1010. 975, 955, 930, 895, 860, 840, 815 cm^{-1}.

Attempted acetylation of monotosylate. Tetraacetyldihydroverbenalin monotosylate (90 mg) was dissolved in pyridine (0·9 ml) and acetic anhydride (0·15 ml). After 24 hr at room temp, the solution was added to 5% sulfuric acid and extracted with methylene chloride. The extract was dried ($MgSO_4$) and evaporated to yield, after one recrystallization from acetone–hexane, 85 mg starting material, m.p. and mixed m.p. 157–8·5°.

Epi-pentaacetyldihydroverbenalin. Tetraacetyldihydroverbenalin monotosylate (600 mg), tetraethylammonium acetate (1·5 g) and acetone (10 ml) were heated under reflux for 41 hr. The solution was then cooled, evaporated to dryness, the residue dissolved in water and extracted with 4 portions of methylene chloride. The combined extracts were dried ($MgSO_4$) and evaporated to yield an oil which subsequently was heated under reflux with sodium acetate (250 mg) in acetic anhydride (3 ml) during 3 hr. After stirring the solution for a few hrs with water the aqueous emulsion was extracted with methylene chloride. The extract was dried ($MgSO_4$) and evaporated to yield an oil (310 mg) which crystallized from 75% aqueous methanol. Four recrystallizations from methanol yielded 81 mg product, m.p. 118–27°. Five additional recrystallizations from methanol failed to improve the m.p. The mother liquor from the seventh recrystallization (17 mg, m.p. 111–127°) was recrystallized 3 times from acetone–hexane–ether to yield 5 mg material, m.p. 134–38°. Mixed m.p. with loganin pentaacetate (m.p. 137–138°) was 118–22°.

IR spectrum (KBr): 1750, 1705, 1635, 1440, 1375, 1285, 1230, 1165, 1090, 1070, 1045, 1000, 955, 910, 870, 800, 775, 740 $cm.^{-1}$ UV spectrum (ethanol): λ_{max} 234 mμ (ε 11,500). (Found: C, 54·08; H, 5·85; Calc. for $C_{27}H_{36}O_{15}$: C, 53·99; H, 6·05%).

Acetylation of tetraacetyldihydroverbenalin. Tetraacetyldihydroverbenalin (180 mg), acetylated in pyridine–acetic anhydride in the usual manner furnished after one recrystallization from 70% aqueous ethanol 165 mg product. Two recrystallizations from acetone–hexane yielded material with m.p. 172–174°, pure and mixed with authentic pentaacetyldihydroverbenalin.

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