TABLE III

PARTITION CHROMATOGRAPHY OF *Senecio ambrosioides* EXTRACT

Fractions	R_f value	Residue, mg.	M.p., °C.
1–32		None	
33–37	0.62	65	243–245
38	.58, 0.62	15	
39–43	.58	10	215–217
44–47		None	
48–54	.44	8	207–208

ing constants; m.p. 212–220°; rotation, 0.0296 g. made up to 2.2 ml. in chloroform at 27° gave α_D −0.96°, l 1; $[\alpha]^{27}_D$ −71.6°; R_f 0.44, 0.58, 0.62. *Anal.* Found: C, 64.37; H, 7.74; N, 4.13.

Partition Chromatography of Crude Extract from *Senecio ambrosioides*.—A chromatographic column using 60 g. of Celite 545 and 100 mg. of crude extract prepared in the usual way was placed on top of the column. Elution was performed with mixtures of carbon tetrachloride and chloroform as follows: 4:1 for fractions 1–30; 3:2 for fractions 32–38; 2:3 for fractions 39–45; pure chloroform for fractions 46–60. The results are indicated in Table III.

The product from fractions 33–37 was identified as senecionine. Fractions 39–43 contained seneciphylline and fractions 48–54 retrorsine. These were all recognized by determination of melting points and comparison of the infrared spectra with those of authentic samples.

URBANA, ILLINOIS

[CONTRIBUTION FROM THE MCPHERSON CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

Bitter Principles of Plants. I. Columbin: Preliminary Structural Studies[1]

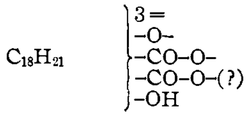
BY M. P. CAVA AND E. J. SOBOCZENSKI

RECEIVED MAY 7, 1956

The major bitter principle of columbo root, columbin, and a number of its transformation products have been investigated. On the basis of spectral and hydrogenation studies a number of conclusions have been reached concerning the nature of the functional systems in the columbin molecule and their interrelations.

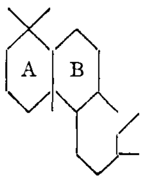
Among the chemical compounds present in the root of the columbo tree, *Jateorrhiza palmata*, is the nitrogen-free bitter principle columbin (I). Although first isolated by Wittstock in 1830,[2] it was only in 1936 that a pure sample was reported by Wessely,[4c] who determined its molecular formula to be $C_{20}H_{22}O_6$. A series of papers by Feist[3] and by Wessely[4] contain the major previous contributions to the study of the columbin molecule.

The most important results of the earlier work may be summarized briefly as follows. Columbin (I), $C_{20}H_{22}O_6$, is isomerized easily by base to isocolumbin (II), $C_{20}H_{22}O_6$. Both columbin and isocolumbin are acetylated by vigorous treatment with sodium acetate in acetic anhydride to the same monoacetyl derivative, acetylisocolumbin (III), $C_{22}H_{24}O_7$. A methylation product, methylisocolumbin (IV), $C_{21}H_{24}O_6$, is produced[5] by methyl sulfate and alkali treatment of columbin or isocolumbin. Although compounds I–IV are all *neutral* substances, they possess the remarkable property of losing carbon dioxide upon melting to give, respectively, decarboxycolumbin (V), $C_{19}H_{22}O_4$, decarboxyisocolumbin (VI), $C_{19}H_{22}O_4$, decarboxyacetylisocolumbin (VII), $C_{21}H_{24}O_5$, and decarboxymethylisocolumbin (VIII), $C_{20}H_{24}O_4$. Columbin and decarboxycolumbin, as well as their iso derivatives, absorb four moles of hydrogen on catalytic reduction to give saturated monocarboxylic acids, *e.g.*, columbin gives octahydrocolumbic acid (IX), $C_{20}H_{30}O_6$. Equivalent weight titrations indicated that the carboxyl group of the octahydro acids arose from hydrogenolysis of a lactone ring.[4a] A second difficultly opened lactone ring was at first suspected in the undecarboxylated compounds, but this hypothesis was later rejected.[4c] In addition, the sixth oxygen atom of columbin was assumed to be present as part of a stable ether ring. These results may be summarized in the partial structure for columbin (Ia)

(Ia) 

$$C_{18}H_{21}\left\{\begin{array}{l}3=\\-O-\\-CO-O-\\-CO-O-(?)\\-OH\end{array}\right.$$

Zinc dust distillation of columbin gave 1,2,5-trimethylnaphthalene, strongly suggesting that columbin is a member of the class of diterpenes possessing the sclareol type skeleton X



X

Our examination of columbin began with a study of its infrared spectrum. The difficultly acetylatable hydroxyl appeared at 2.86 μ; in addition, two carbonyls (5.73 and 5.80 μ) were observed. As the subsequent arguments will show, both carbonyls represent lactone rings, which we shall designate as lactones L-1 and L-2, respectively.

When columbin is completely reduced to octahydrocolumbic acid (XI), lactone L-1 (5.73 μ) is unchanged. It must represent, both before and after reduction, an unconjugated δ-lactone.[6] The possibility of attributing this carbonyl to a cyclopentanone structure is discounted by the absence of a weak saturated ketone band in the ultraviolet in

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[1] From the Ph.D. Dissertation of E. J. Soboczenski, The Ohio State University, 1956.

[2] Wittstock, *Poggendorfs Ann.*, **19**, 298 (1830).

[3] K. Feist, *et al.*, (a) *Ann.*, **517**, 119 (1935); (b) **519**, 124 (1935); (c) **521**, 184 (1935); (d) **522**, 185 (1936); (e) **523**, 289 (1936); (f) **534**, 41 (1938).

[4] F. Wessely, *et al.*, (a) *Monatsh.*, **66**, 87 (1935); (b) *Ann.*, **522**, 41 (1936); (c) *Monatsh.*, **68**, 313 (1936); (d) **70**, 30 (1937).

[5] The yield of methylisocolumbin is low, water-soluble salts and unreacted isocolumbin being the major reaction products.

[6] L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1954.

the 290 mμ region.[7] Lactone L-2 (5.80 μ) of columbin is no longer present in octahydrocolumbic acid: it has been replaced by the new carboxylic acid function (5.86 μ). This conversion represents a hydrogenolysis of the L-2 lactone ring. The long wave length absorption of L-2 (5.80 μ) suggests[6] that it is an α,β-unsaturated δ-lactone. In agreement with this formation, columbin shows strong end absorption in the ultraviolet, with an apparent maximum at 209 mμ (log E 3.78). It has been shown that α,β-unsaturated esters absorb strongly in this region.[8]

The base-catalyzed isomerization of columbin to isocolumbin involves a different type of change in the L-2 lactone system. In isocolumbin (II) L-2 is no longer α,β-unsaturated but, like L-1, absorbs as an unconjugated δ-lactone at 5.73 μ.[9] The ultraviolet spectrum of isocolumbin (and its derivatives) still shows, however, an apparent maximum at 209 mμ (log E 3.80), indicating the formation of a new chromophore system as a result of the columbin–isocolumbin transformation. The probable nature of this chromophore will be pointed out later in this discussion.

Methylisocolumbin, like isocolumbin, shows only one carbonyl (L-1 and L-2) at 5.73 μ[9]; the hydroxyl group (2.86 μ) is not present in methylisocolumbin, having been converted to a methoxy group. The hydrogenation product of methylisocolumbin, octahydromethylisocolumbic acid (XII), $C_{21}H_{32}O_6$, contains a lactone ring (L-1, 5.73 μ) and a carboxyl group (5.86 μ), formed by hydrogenolysis of the isomerized (5.73 μ) lactone L-2. The changes discussed up to this point are summarized in Chart I

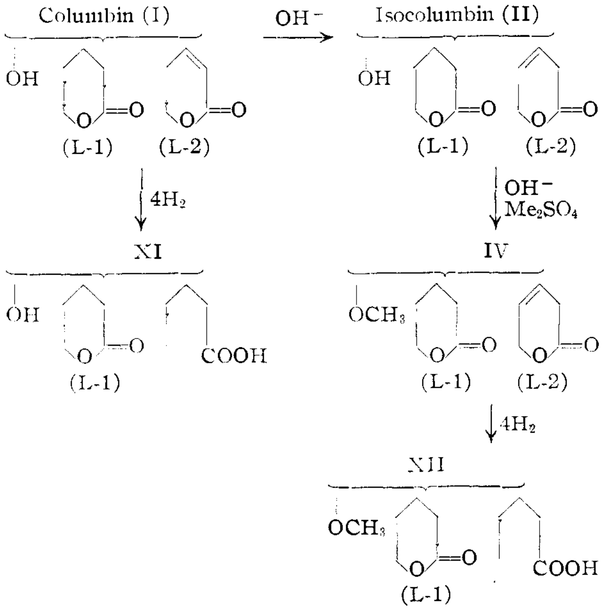
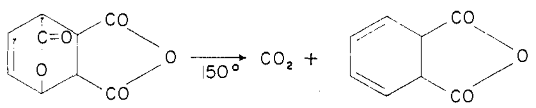


CHART I

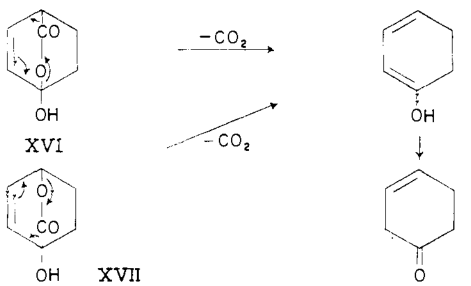
The most interesting reaction of columbin is its very facile decarboxylation upon melting to yield carbon dioxide and decarboxycolumbin (V). Unlike the previously considered changes, this reaction does not involve lactone L-2, which is present (5.80 μ) in decarboxycolumbin and, indeed, still able to undergo its usual hydrogenolysis on catalytic reduction with the production of octahydrodecarboxycolumbic acid (XIII). However, lactone L-1 (5.73 μ) is *no longer present* in decarboxycolumbin, having been *eliminated as carbon dioxide*. As a result of the decarboxylation, the alcoholic hydroxyl (2.86 μ) of columbin also has vanished, to be replaced by a new carbonyl group absorbing at 5.85 μ, characteristic of an unconjugated acyclic ketone or cyclohexanone. In confirmation of the ketonic nature of this carbonyl, it exhibits a weak ultraviolet maximum (log E 1.60) at 290 mμ.[7] The ketone group is quite hindered, however, since it gives no derivatives with the usual carbonyl reagents.

The ready decarboxylation of the saturated δ-lactone (L-1) of columbin is a most unusual reaction and must require very special structural features in the vicinity of lactone L-1. We believe that this reaction is analogous to the decarboxylation of the maleic anhydride α-pyrone adduct (XIV),[10] when formed at 150°, to carbon dioxide and 1,2-dihydrophthalic anhydride (XV)



XIV XV

The presence of this type of decarboxylation system in columbin could give rise to the unconjugated ketone group of decarboxycolumbin with disappearance of the original columbin hydroxyl, if the latter were, in columbin, attached to one of the bridgehead positions of lactone L-1, as in XVI or XVII



Columbin is indeed a tertiary alcohol: it is unchanged by chromic oxide in pyridine solution, a selective oxidant[11] for primary and secondary alcohols. As a tertiary alcohol, it is unusually resistant to dehydration, in accord with a bridgehead alcohol formulation. Drastic treatment with acetic anhydride–sodium acetate esterifies the alcoholic group, no dehydration taking place.

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(7) L. Dorfman, *Chem. Revs.*, **53**, 47 (1953).

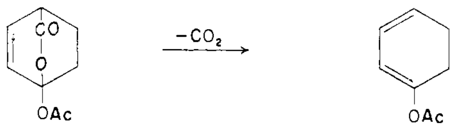
(8) J. L. H. Allen, E. R. H. Jones and M. C. Whiting, *J. Chem. Soc.*, 1862 (1955).

(9) Only one carbonyl band is seen in methylene chloride solution; in Nujol, the two lactone bands are resolved.

(10) O. Diels and K. Alder, *Ann.*, **490**, 257 (1931); XV was isolated as its adduct with a second mole of maleic anhydride. A further study of XIV has been made by R. B. Woodward and B. Landau (B. Landau, Ph.D. Thesis, Harvard University). The decarboxylation of a lactone analogous to XIV has been postulated by H. Conroy (THIS JOURNAL, **74**, 3046 (1952)) as the final step in the degradation of picrotoxin to picrotoxadiene.

(11) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

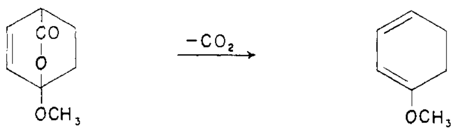
A choice between the structural types XVI and XVII can be made on the basis of the infrared spectrum of acetylisocolumbin. This substance shows, in addition to the lactones L-1 and L-2 (5.73 μ), the new ester carbonyl of the acetate group at 5.66 μ. Ordinarily one would be tempted to characterize this acetate as an enol acetate. The completely saturated acid corresponding to acetylisocolumbin, octahydroacetylisocolumbic acid (XVIII), $C_{22}H_{32}O_7$, still exhibits the 5.66 μ band, ruling out this possibility. Saturated 1,1-alkylidene esters, however, absorb in the same region as enol esters: the value 5.68 μ is reported[6] for 1,1-diacetoxypropane. The spectrum of acetylisocolumbin is, therefore, consistent with partial structure XIX,[12] corresponding to XVI for columbin itelf



XIX XX

It must follow then that, unlike decarboxycolumbin, decarboxyacetylisocolumbin (VII) is not a ketone but rather an *enol acetate containing a cyclohexadiene system* as in XX. This conclusion is entirely verified by the spectral evidence. In the infrared, the dienol acetate and the isomerized lactone L-2 appear at 5.73 μ; in addition, a band at 6.33 μ is present, characteristic of a conjugated double bond in a diene system.[6] In the ultraviolet a strong maximum (log E −3.70) is found at 271 mμ. The calculated maximum for an acetoxycyclohexadiene containing one additional diene substituent (no exo double bond) is 268 mμ. An additional substituent (or exo double bond) would raise the calculated value to 273 mμ.[13]

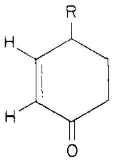
The decarboxylation of methylisocolumbin to decarboxymethylisocolumbin (VIII) involves the transformation of the pseudoester system XXI to the dienol ether XXII



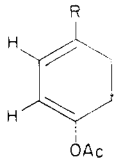
XXI XXII

Decarboxymethylisocolumbin is very similar spectrally to decarboxyacetylisocolumbin. The infrared spectrum shows bands at 5.73 μ (isomerized lactone L-2) and at 6.30 μ (conjugated diene). The ultraviolet spectrum shows a maximum (log E 3.73) at 278 mμ, 7 mμ longer than in decarboxyacetylisocolumbin. The bathochromic shift calculated for the replacement of an acetoxy group by alkoxy in a diene system is 6 mμ.[7]

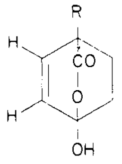
Decarboxyisocolumbin (VI) is obtained readily by decarboxylation of isocolumbin, and shows the expected carbonyl absorption bands at 5.73 μ (isomerized lactone L-2) and at 5.85 μ (cyclohexanone system). It has been said to be produced also[4a] by alkali treatment of decarboxyacetylisocolumbin or decarboxycolumbin. We have found, however, that decarboxyisocolumbin itself is *not stable to base*, but is easily converted to a new isomer, ψ-decarboxyisocolumbin (XXIII), $C_{19}H_{22}O_4$. This compound, which exhibits carbonyls at 5.73 μ (isomerized lactone L-2) and at 5.96 μ (α,β-unsaturated ketone), is the *conjugated cyclohexenone* corresponding to decarboxyisocolumbin. Its ultraviolet absorption spectrum shows a maximum (log E 3.80) at 230 mμ, indicating[13] that *both carbons of the cyclohexenone double bond are unsubstituted,* as in part structure XXIV.[14] The enol acetate, decarboxyacetylisocolumbin, cannot then bear more than *one* alkyl substituent on its diene system, as in XXV. Columbin itself must be partially represented as XXVI, since its cyclohexene double bond occupies the *same position* as it does in ψ-decarboxyisocolumbin



XXIV

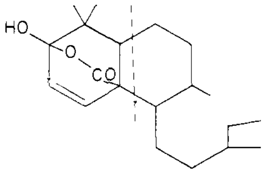


XXV

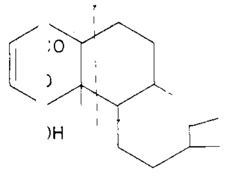


XXVI

If we assume, as very likely, that columbin contains the sclareol skeleton, then only ring A of this skeleton X can correspond to the columbin bridged lactone system XXVI. Two possible structural types can be constructed using the sclareol A ring: both of these, XXVII and XXVIII, are equally consistent with the spectral data discussed above.



XXVII



XXVIII

Although XXVII cannot be definitely discarded, XXVIII is considerably more attractive at present for several reasons. It explains more easily the formation of 1,2,5-trimethylnaphthalene on aromatization; it also shows the carbonyl of lactone L-1 as derived from a carboxyl group in a position in which it appears in all of the known diterpene acids as well as the lactone marrubiin. Finally, we have been unable to find consistent spectral evidence in the columbin derivatives studied for the

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[12] It has been pointed out by a referee that the acetate of structure XVII might be expected to show carbonyl absorption at a much lower than normal wave length. However, we have examined the spectrum of ethyl acetoxyacetate and found that the two carbonyls of this model substance absorb at 5.72 and 5.75 μ, *i.e.*, within the normal ester range.

[13] L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd edition, Reinhold Pub. Corp., New York, N. Y., 1949, pp. 185–198.

[14] It has been stated on the basis of Woodward's observations on the ultraviolet spectra of α,β-unsaturated ketones, that α- or β-monosubstituted α,β-unsaturated ketones (Class I) absorb at 225 ± 5 mμ and that α- or β-disubstituted α,β-unsaturated ketones (double bond not exo to a ring) (Class II) absorb at 235 ± 5 mμ. (See ref. 7, and F. A. Miller, in "Organic Chemistry," Vol. III, ed., H. Gilman, John Wiley and Sons, New York, N. Y., 1953, p. 168.) If this were true then our observed value of 230 mμ for XXIII would be exactly half way between the Class I and Class II ketones. Close inspection of Woodward's data (R. B. Woodward, THIS JOURNAL, **63**, 1123 (1941); **64**, 76 (1942)) shows *no example* of a Class II ketone absorbing below 235 mμ. We hope to expound more fully upon this common misinterpretation of Woodward's Rules in a subsequent publication.

presence of a *gem*-dimethyl group as a doublet at 7.2–7.3 μ.[6]

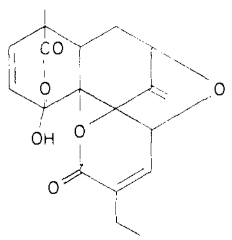
Of the three ethylenic double bonds present in columbin, only two are detectable spectrally. One is the double bond conjugated with lactone L-2 which shifts its carbonyl to 5.80 μ; the second absorbs directly at 11.4 μ. The third unobservable double bond is simply the cyclohexene double bond of ring A of columbin. This double bond can be *selectively reduced* catalytically using palladium charcoal at 0°. The resulting dihydrocolumbin (XXIX), $C_{20}H_{24}O_6$, has an infrared spectrum practically indistinguishable from that of columbin itself. Unlike columbin, however, dihydrocolumbin melts sharply and *does not decarboxylate* upon heating. This behavior is completely consistent with a structure in which the cyclohexene double bond has been reduced, since a cyclic decarboxylation mechanism (*e.g.*, as in XIV) can no longer operate. Dihydrocolumbin is converted by further reduction to a pure octahydrocolumbic acid; acetylation of dihydrocolumbin by acetic anhydride–sodium acetate gives dihydroacetylisocolumbin (XXX), $C_{22}H_{26}O_7$ (acetoxy at 5.66 μ, lactones L-1 and L-2 at 5.73 μ). These experiments prove that the two double bonds remaining in dihydrocolumbin are those which control the processes of hydrogenolysis and isomerization of lactone L-2.

The position of the double bond which moves out of conjugation with lactone L-2 in going from the columbin to the isocolumbin series is irrelevant to the hydrogenolysis of lactone L-2: columbin and isocolumbin *both* are reducible to carboxylic acids. The double bond essential for the hydrogenolysis process is that observed at 11.4 μ. It is present in all columbin and isocolumbin derivatives, *except the octahydro acids.* Since lactone L-2 is not an enol lactone (or even α,β-unsaturated in the isocolumbin series), hydrogenolysis of this ring is explicable only if the 11.4 μ double bond is situated at a position *allylic to the ester oxygen* of lactone L-2.[15]

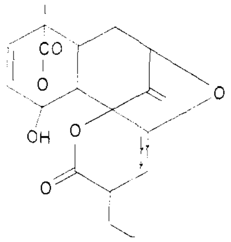
The terminal methylene group $>C{=}CH_2$ generally exhibits a strong band in the infrared very close to 11.2 μ.[6] Several cases are known, however, such as camphene and longifolene,[16] in which a methylene group exo to a somewhat strained bridged ring absorbs at 11.4 μ. A similar situation probably exists in columbin, the methylene group being exo to a tetrahydrofuran ring containing the inert oxygen atom. No formaldehyde has yet been obtained by ozonolysis of columbin. This fact does not, however, prove the absence of a methylene group, since several cases are known (*e.g.*, longifolene[16]) in which ozonolysis of a methylene function gives only insignificant yields of formaldehyde.

The postulation of a tetrahydrofuran ring in columbin offers an explanation of the strong absorbtion of isocolumbin at 209 mμ and, indirectly, of the columbin–isocolumbin transformation. It is known[17] that a double bond may move readily out of conjugation with a carbonyl group into conjugation with an *ether oxygen* under the influence of alkali. Enol ethers generally show only weak absorption in the 210 mμ region. When the ether oxygen is part of a tetrahydrofuran ring, however, the absorption in this region is very high,[18] as is observed for isocolumbin. In the infrared, both columbin and isocolumbin show a rather weak band at 6.25 μ, attributable to the conjugated double bond both before and after base isomerization.

Although a complete structure for columbin has by no means been completely established on the basis of present evidence, the possibilities are severely limited if the sclareol skeleton is indeed present, as in XXVIII. One is tempted to conclude that lactone L-2 is a spiro ring derived from the long side chain attached to ring B of the sclareol skeleton X and that the allylic methylene is the one carbon substituent of ring B. A possible structure for columbin accommodating all of the known facts is illustrated in XXXI,[19] isocolumbin then being represented as XXXII. We have accepted these structures as at least a reasonable working hypothesis pending the accumulation of further degradative evidence.



XXXI



XXXII

NOTE ADDED IN PROOF.—Since the acceptance of this manuscript for publication two papers have appeared (D. H. R. Barton and D. Elad, *J. Chem. Soc.*, 2085, 2090 (1956)) describing evidence which seems to establish completely the structure of columbin. The decarboxylation system proposed by us is confirmed, the hydroxyl being at the opposite bridgehead, however, as in XVII, the sclareol skeleton being not normal, but *rearranged.* Furthermore, despite the large spectral shift of 0.07 μ in lactone L-2 on going from columbin to isocolumbin, this change involves merely an epimerization, *both* double bonds associated with L-2 being part of the allylic system in the form of a *furan* ring (*i.e.*, the 11.4 μ and 209 mμ chromophore).

Experimental[20,21]

Columbin (I).—Powdered columbo root (S. B. Penick and Co., 200 lb.) was extracted continuously with tetrahydrofuran for 3 days. The dark extract was concentrated to a small volume, Skellysolve C was added, and a further quantity of solvent distilled over to remove the last portion of tetrahydrofuran present. The Skellysolve solution was decanted from the insoluble gummy residue and the latter dissolved in hot ethanol. On standing, the ethanol solution slowly deposited crude columbin (200 g.) as yellowish crystals. Recrystallization from methanol (charcoal) gave white needles. These were taken up in chloroform, the

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[15] W. G. Dauben and P. D. Hance, THIS JOURNAL, **77**, 606 (1955).

[16] P. Naffa and G. Ourisson, *Chem. & Ind.*, 917 (1953).

[17] L. N. Owen and M. U. S. Sultanbawa, *J. Chem. Soc.*, 3098 (1949).

[18] G. Eglinton, E. R. H. Jones and M. C. Whiting, *ibid.*, 2873 (1952).

[19] The position of the ethyl side chain of lactone L-2 in this formula is in accord with the known formation of acetic and butyric acids on heating columbin with 50% alkali.[3b]

[20] Melting points are uncorrected. Analyses were carried out by Galbraith Laboratories, Knoxville, Tennessee.

[21] The melting points of those compounds which decarboxylate on melting are not sharp and vary slightly with the heating rate.

chloroform-insoluble portion (chasmanthin[4b]) was filtered off, and the filtrate evaporated. Crystallization of the residue from methanol gave columbin, m.p. 192–195° dec. Wessely has reported[4c] m.p. 201° dec. for his purest columbin sample. Further crystallization of our material, or purification by the partition method of Wessely[4b] did not raise the melting point above 195°.

Infrared spectrum (**CH_2Cl_2**): bands at 2.82 (hydroxyl); 5.73 μ (δ-lactone); 5.80 μ (α,β-unsaturated δ-lactone); 11.40 μ (terminal methylene). In Nujol mull the lactone bands appeared at 5.72 and 5.87 μ, respectively.[22]

Ultraviolet spectrum (**EtOH**): no maxima above 209 mμ; apparent maximum at 209 mμ (log *E* 3.78).

Treatment of Columbin with Pyridine–Chromic Oxide.—Columbin (0.500 g.) was added to a solution of chromic oxide (0.420 g.) in pyridine (10 ml.) at 0°. After standing overnight at room temperature the solution was poured into a cold mixture of water (50 ml.) and concentrated hydrochloric acid (12 ml.). The mixture was extracted with methylene chloride and the organic layer washed with water, evaporated and the residue crystallized from ethanol–water to yield recovered columbin (0.415 g., 83%), identical with the starting material.

Dihydrocolumbin (XXIX).—A solution of columbin (2.00 g.) in acetone (60 ml.) containing 10% palladium-charcoal (0.40 g.) was hydrogenated at 0° at atmospheric pressure for 31 min. when one molar equivalent of hydrogen had been absorbed. The reduction was stopped and the catalyst filtered off with the aid of celite. Evaporation of the filtrate and crystallization of the residue from methanol gave dihydrocolumbin (1.62 g., 80%), m.p. 232–234°. A completely pure product was obtained by dissolving the above material in methylene chloride and chromatographing it on alumina (Brockman I, *p*H 7.5). Elution of the column with methylene chloride, evaporation of the eluate and crystallization of the residue from methanol gave an 82% recovery of XXIX, m.p. 239–240.5°.

Anal. Calcd. for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71. Found: C, 66.69; H, 6.51.

Infrared spectrum (**CH_2Cl_2**): bands at 2.82 μ (hydroxyl); 5.73 μ (δ-lactone); 5.80 μ (α,β-unsaturated δ-lactone); 11.40 μ (terminal methylene). In Nujol mull the lactone bands appeared at 5.72 and 5.87 μ, respectively.

Ultraviolet spectrum (**EtOH**): no maximum above 211 mμ; log E_{211} 3.76.

Octahydrocolumbic Acid (IX). A. From Dihydrocolumbin.—A solution of dihydrocolumbin (0.299 g.) in methanol (25 ml.) was hydrogenated at 28° at atmospheric pressure in the presence of 10% palladium-charcoal (0.200 g.). Reduction ceased after 16 min., when 2.91 molar equivalents of hydrogen had been absorbed. The catalyst was removed by filtration with the aid of celite, the filtrate was concentrated to a small volume and saturated aqueous sodium bicarbonate was added. After washing with methylene chloride to remove neutral impurities, the aqueous solution was acidified with hydrochloric acid and again extracted with methylene chloride. Evaporation of the organic phase and crystallization of the residue from aqueous methanol gave octahydrocolumbic acid (0.237 g., 78%), m.p. 158–160°.

Anal. Calcd. for $C_{20}H_{30}O_6$: C, 65.55; H, 8.25; neut. equiv., 366. Found: C, 65.24; H, 8.01; neut. equiv., 372; tetranitromethane test, negative.

Infrared spectrum (**CH_2Cl_2**): bands at 5.74 μ (δ-lactone) and 5.87 μ (carboxylic acid).

B. **From Columbin.**—A solution of columbin (1.00 g.) in methanol (75 ml.) was hydrogenated at 32° at atmospheric pressure in the presence of 10% palladium–charcoal (0.500 g.). After 60 min. 3.63 molar equivalents of hydrogen had been absorbed and hydrogenation ceased. Isolation of the acid as described above (method A) gave 0.473 g. (45%), m.p. 158–160°. This material was identical in all respects with that prepared by reduction of dihydrocolumbin. The previously reported octahydrocolumbic acid was noncrystalline.[4c]

Isocolumbin (II).—Columbin (5.12 g.) was heated on the steam-bath with a mixture of methanol (45 ml.), 5% aqueous sodium hydroxide (20 ml.) and water (25 ml.). After 5 min. the clear solution was cooled and extracted twice with methylene chloride (20 ml.). The aqueous phase was filtered, acidified to *p*H 2 with hydrochloric acid, and extracted thoroughly with methylene chloride. The dried organic phase was concentrated to 15 ml. and methanol (70 ml.) added. Crystalline isocolumbin (4.137 g., 81%), m.p. 183–187° dec., separated. A further quantity was easily obtained from the mother liquor. The previously reported melting point was around 190° dec.[4a]

Infrared spectrum (**CH_2Cl_2**): bands at 2.85 μ (hydroxyl); 5.74 μ (δ-lactones); 11.40 μ (terminal methylene). In Nujol mull the lactones appeared at 5.73 and 5.80 μ.

Ultraviolet spectrum (**EtOH**): no maxima above 209 mμ; apparent maximum at 209 mμ (log *E* 3.80).

Hydrogenation of Isocolumbin. A. Dihydroisocolumbin.—A solution of isocolumbin (0.500 g.) in ethyl acetate (50 ml.) was hydrogenated at 0° at atmospheric pressure in the presence of 10% palladium-charcoal. After 15 min. one molar equivalent of hydrogen had been absorbed. The solution, after filtration through celite, was evaporated and the residue crystallized from methanol to yield white needles (0.400 g., 80%), m.p. 234–235° (no decomposition), after losing methanol of crystallization at 107–108°.

Anal. (1) Air dried: Calcd. for $C_{20}H_{24}O_6 \cdot CH_3OH$: C, 64.27; H, 7.19. Found: C, 64.42; H, 7.29. (2) Dried at 110° for 36 hr.: Calcd. for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71. Found: C, 66.48; H, 6.60.

Infrared spectrum (**methanolate, $CHCl_3$**): bands at 2.77 and 2.85 μ (hydroxyls); 5.73 μ (δ-lactones); 11.40 μ (terminal methylene).

B. **Octahydroisocolumbic Acid.**—A solution of dihydroisocolumbin (0.652 g.) in methanol (75 ml.) was hydrogenated at 30° at atmospheric pressure in the presence of 10% palladium–charcoal (0.300 g.). The acid formed was worked up as in the case of octahydrocolumbic acid. The product obtained (0.360 g., 54%) melted over the range 140–160°, and appeared to be a mixture of stereoisomers.[4c] It gave a negative tetranitromethane test and showed carbonyl bands in chloroform at 5.74 μ (δ-lactone) and 5.86 μ (carboxyl carbonyl).

Decarboxycolumbin (V).—Columbin was pyrolyzed at 220° as previously described.[4a] The crude pyrolysis product was purified readily by passing its solution in benzene through alumina (Brockman IV, neutral), the column being eluted with benzene. Evaporation of the eluate and crystallization of the residue from ethanol gave pure decarboxycolumbin, m.p. 146.8–148.2° (reported[4a] 147.5–149°).

Infrared spectrum (**CH_2Cl_2**): bands at 5.80 μ (α,β-unsaturated δ-lactone); 5.85 μ (saturated ketone); 11.40 μ (terminal methylene).

Ultraviolet spectrum (**EtOH**): λ_{max} 290 mμ (log *E* 1.60); apparent maximum at 210 mμ (log *E* 3.80).

Octahydrodecarboxycolumbic Acid.—A solution of decarboxycolumbin (0.300 g.) in methanol (30 ml.) was hydrogenated at 50° at atmospheric pressure in the presence of 10% palladium–charcoal. After 40 min. 4.0 molar equivalents had been absorbed and hydrogenation ceased. The acid was isolated as in the case of octahydrocolumbic acid. The product (0.254 g., 83%) formed white crystals, m.p. 177–179°, raised by further recrystallization to 179–181° (reported[3f] 186°). The compound gave a negative tetranitromethane test.

Anal. Calcd. for $C_{19}H_{30}O_4$: C, 70.77; H, 9.38; neut. equiv., 322. Found: C, 70.95; H, 9.59; neut. equiv., 320.

Infrared spectrum (**CH_2Cl_2**): band at 5.86 μ (saturated ketone and carboxyl carbonyls).

Ultraviolet spectrum (**EtOH**): λ_{max} 289 mμ (log *E* 1.54).

Decarboxyisocolumbin (VI).—Isocolumbin (1.57 g.) was heated under nitrogen at 240° for 10 min. The resulting yellow glass was dissolved in chloroform and the solution chromatographed on alumina (Brockman IV, neutral), the column being eluted with chloroform. Concentration of the eluate and crystallization of the residue from methanol gave colorless crystals of decarboxyisocolumbin (0.85 g., 62%), m.p. 203–206° (reported[4a] about 210°).

Infrared spectrum (**CH_2Cl_2**): bands at 5.73 μ (δ-lactone); 5.85 μ (saturated ketone); 11.40 μ (terminal methylene).

Ultraviolet spectrum (**EtOH**): λ_{max} 287 mμ (log *E* 1.69).

ψ-Decarboxyisocolumbin (XXIII).—A solution of decarboxycolumbin (1.000 g.) in methanol (50 ml.) containing potassium hydroxide (0.357 g.) was heated at 50° for 15 min.

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[22] The lactone carbonyl absorption band of marrubin exhibits a bathochromic shift of 0.07 μ on going from a chloroform solution to a paraffin mull. See W. Cocker, B. E. Cross, S. R. Duff, J. T. Edwards and T. F. Holley, *J. Chem. Soc.*, 2540 (1953).

The solution was concentrated at room temperature, dilute hydrochloric acid added, and the precipitate extracted into methylene chloride. The solvent was dried over sodium sulfate, evaporated to dryness and the crystalline residue sublimed at 240° (3 mm.). Crystallization of the sublimate from methanol gave ψ-decarboxyisocolumbin (0.880 g., 88%), m.p. 194–197°; further crystallization raised the melting point to 197–200°.

Anal. Calcd. for $C_{19}H_{22}O_4$: C, 72.59; H, 7.05. Found: C, 72.55; H, 7.15.

Infrared spectrum (CH_2Cl_2): bands at 5.74 μ (δ-lactone); 5.96 μ (α,β-unsaturated ketone); 11.40 μ (terminal methylene).

Ultraviolet spectrum (EtOH): λ_{max} 230 mμ (log E 3.80); λ_{max} 327 mμ (log E 1.84).

Basic isomerization of decarboxyisocolumbin proceeded in exactly the same manner as described above to give the same ψ-decarboxyisocolumbin.

Acetylisocolumbin (III).—The acetylation of columbin was best carried out as described by Wessely[4a] to give, in 60–70% yield, acetylisocolumbin, m.p. 225–230° dec. (reported[4a] 230° dec.).

Infrared spectrum (CH_2Cl_2): bands at 5.66 μ (acetyl carbonyl): 5.73 μ (δ-lactones); 11.40 μ (terminal methylene).

Ultraviolet spectrum (EtOH): no maximum above 209 mμ; apparent maximum at 209 mμ (log E 3.80).

Acetylisodihydrocolumbin (XXX).—A mixture of dihydrocolumbin (2.00 g.), anhydrous sodium acetate (2.0 g.) and acetic anhydride (25 ml.) was refluxed for 3 hr. The cooled reaction mixture was poured with stirring into water (200 ml.). The aqueous mixture was then extracted three times with 25-ml. portions of methylene chloride. The organic layer was washed with water, then with saturated sodium bicarbonate solution, dried and evaporated to dryness. The residue was crystallized from methanol, the crystalline acetate (1.51 g.) dissolved in methylene chloride and the resulting solution chromatographed on alumina (Brockman IV, neutral). Elution of the column with methylene chloride, evaporation of the eluate and crystallization of the residue from methanol gave pure acetylisodihydrocolumbin (1.03 g., 46%), m.p. 279–283°.

Anal. Calcd. for $C_{22}H_{26}O_7$: C, 65.16; H, 6.51. Found: C, 65.20; H, 6.40.

Infrared spectrum (CH_2Cl_2): bands at 5.66 μ (acetyl carbonyl); 5.73 μ (δ-lactones); 11.40 μ (terminal methylene).

Octahydroacetylisocolumbic Acid (XVIII).—A solution of acetylisodihydrocolumbin (0.400 g.) in acetone (50 ml.) was hydrogenated at room temperature in the presence of 10% palladium charcoal (0.200 g.). After 24 min. 2.8 molar equivalents of hydrogen had been absorbed and hydrogenation ceased. The acid formed was isolated as in the preparation of octahydrocolumbic acid. Recrystallization of the crude product from methanol gave octahydroacetylisocolumbic acid (0.302 g., 74%), m.p. 226–228°. The melting point was raised by a second crystallization to 229–230°. The acid gave a negative tetranitromethane test.

Anal. Calcd. for $C_{22}H_{32}O_7$: C, 64.68; H, 7.90. Found: C, 64.67; H, 7.76.

Infrared spectrum (CH_2Cl_2): bands at 5.67 μ (acetyl carbonyl); 5.73 (δ-lactone); 5.86 μ (carboxyl carbonyl).

Decarboxyacetylisocolumbin (VII).—Acetylisocolumbin (0.800 g.) was pyrolyzed at 240° for 5 min., after which time carbon dioxide ceased to be evolved. The residual glass was sublimed at 260° (0.5 mm.) and the sublimate (0.641 g.) crystallized from methanol to give pure decarboxyacetylisocolumbin (0.500 g., 70%), m.p. 162–163.5° (reported[4a] 169°).

Infrared spectrum (CH_2Cl_2): bands at 5.73 μ (δ-lactone, dienol acetate); 11.40 μ (terminal methylene).

Ultraviolet spectrum (EtOH): λ_{max} 271 mμ (log E 3.70).

Hydrolysis of decarboxyacetylisocolumbin with warm dilute alkali gave ψ-decarboxyisocolumbin, identical in melting point and infrared spectrum with that obtained from alkaline treatment of decarboxycolumbin.

Methylisocolumbin (IV).—The methylation of columbin by methyl sulfate and alkali was carried out as described by Wessely.[4d] The crude product (65% by weight of the starting columbin) contained considerable isocolumbin, as shown by infrared examination. Repeated recrystallization from methanol gave, in 25% yield, pure methylisocolumbin (no hydroxyl in the infrared), m.p. 225–229° dec. (reported[4d] 225° dec.).

Infrared spectrum (CH_2Cl_2): 5.73 μ (δ-lactones); 11.40 μ (terminal methylene). In Nujol mull carbonyl bands appeared at 5.73 and 5.80 μ.

Ultraviolet spectrum (EtOH): no maximum above 209 mμ; apparent maximum at 209 mμ (log E 3.80).

Octahydromethylisocolumbic Acid.—A solution of methylisocolumbin (0.169 g.) in methanol (25 ml.) was hydrogenated at room temperature in the presence of 10% palladium–charcoal (0.084 g.). Hydrogenation ceased after 160 min. when 3.9 molar equivalents of hydrogen had been absorbed. The acid formed was isolated as in the preparation of octahydrocolumbic acid and, after recrystallization to constant melting point, octahydromethylisocolumbic acid (0.080 g., 47%), m.p. 182.5–185°, was obtained. The acid gave a negative tetranitromethane test.

Anal. Calcd. for $C_{21}H_{32}O_6$: C, 66.30; H, 8.48. Found: C, 66.01; H, 8.75.

Infrared spectrum (CH_2Cl_2): bands at 5.72 μ (δ-lactone); 5.88 (carboxyl carbonyl).

Decarboxymethylisocolumbin (VIII).—Methylisocolumbin (0.437 g.) was heated at 240° for 10 min., after which time carbon dioxide was no longer evolved. The resulting brown glass was dissolved in benzene and the solution chromatographed on alumina (Brockman III, neutral). The column was eluted with benzene, the eluate evaporated, and the residue crystallized to constant melting point from methanol to give decarboxymethylisocolumbin (0.055 g., 14%) as colorless needles, m.p. 198.5–200° (reported[4d] 205–206°).

Infrared spectrum (CH_2Cl_2): bands at 5.73 μ (δ-lactone); 11.40 μ (terminal methylene). In Nujol the lactone carbonyl appeared at 5.80 μ.

Ultraviolet spectrum (EtOH): λ_{max} 278 μ (log E 3.73).

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