408. *Colombo Root Bitter Principles. Part I. The Functional Groups of Columbin.*

By D. H. R. Barton and Dov Elad.

Columbin, the most important bitter principle of Colombo root, has been shown to have a $\beta\gamma$-unsaturated α-hydroxy-δ-lactone system (*A*). The $\beta\gamma$-unsaturation has been characterised as the group $-CH{=}CH-$ contained in a six-membered ring. Columbin also possesses a β-substituted furan ring and has a second γ- or δ-lactone system (*B*), which is saturated and bears epimerisable hydrogen α- with respect to its carbonyl group. Lactone system (*B*) is readily cleaved by hydrogenolysis and, for this and other reasons, must be secured with its alkyl oxygen α- with respect to the β-position of the furan ring. Lactone system (*A*) is responsible for the easy decarboxylation of columbin and many of its derivatives.

The root of *Jatrorrhiza palmata* Miers (Colombo root) contains three neutral bitter principles: columbin, $C_{20}H_{22}O_6$, chasmanthin, $C_{20}H_{22}O_7$, and the isomeric palmarin. These compounds have been the subject of prolonged investigation, especially by Wessely [1] and by Feist [2] and their respective collaborators. As yet, however, there has been no agreement as to the nature of the functional groups. The present paper re-analyses the extensive experimental evidence [1,2] and makes definite proposals, confirmed by subsequent investigation, as to the nature of the functional groups in the molecule of columbin.

The principal facts that must be accommodated are as follows. Columbin is easily isomerised by mild treatment with alkali to *iso*columbin. Both columbin and *iso*columbin behave as dilactones towards alkali. However, the consumption of the first mol. of alkali is reversible, that of the second is irreversible and leads to no defined product. On hydrogenation both columbin and *iso*columbin afford octahydro-derivatives. The hydrogenation involves hydrogenolysis, for both products are carboxylic acids; they appear (by titration) to contain no lactone group. Both columbin and *iso*columbin possess one hydroxyl group, for on treatment with acetic anhydride–sodium acetate they afford the same monoacetate. Since this is a derivative of *iso*columbin it should be known as *iso*columbin acetate not columbin acetate as hitherto.[1,2] On methylation with dimethyl sulphate and alkali both columbin and *iso*columbin furnish the same monomethyl ether. This again should be designated *O*-methyl*iso*columbin not [1,2] *O*-methylcolumbin. The hydroxyl group of columbin must, because of these reactions, be somewhat acidic; it is, however, not enolic or phenolic.[1,2]

Both columbin and *iso*columbin lose one mol. of carbon dioxide on melting and afford decarboxycolumbin and decarboxy*iso*columbin, respectively. These decarboxy-compounds cannot be acetylated or methylated and therefore the decarboxylation destroys the original hydroxyl group. Nevertheless, both *iso*columbin acetate and *O*-methyl*iso*columbin also lose one mol. of carbon dioxide at the melting point. Decarboxycolumbin and decarboxy*iso*columbin still retain one lactone grouping, as judged by titration.

If one adopts the hypothesis that columbin contains two lactone groups (*A*) and (*B*), then one, group (*A*), must be lost on decarboxylation. Now hydrogenation of decarboxycolumbin affords decarboxyoctahydrocolumbinic acid and therefore it is the second lactone group, (*B*), which is hydrogenolysed. If one assumes that it is lactone (*B*) which is also hydrogenolysed in the hydrogenation of both columbin and *iso*columbin, then one must explain why the hydrogenation products of these compounds do not titrate as lactones and are not readily decarboxylated. In fact if lactone (*A*) is retained it must be changed in some

[1] (*a*) Wessely, Dinjaski, Isemann, and Singer, *Monatsh.*, 1935, **66**, 87; earlier work is cited also. (*b*) Wessely, Isemann, Singer, and Schönol, *Annalen*, 1936, **522**, 41; Wessely, Schönol, and Isemann, *Monatsh.*, 1936, **68**, 21; Wessely, Münster, and Schönol, *ibid.*, p. 313; Wessely and Jentzsch, *ibid.*, 1937, **70**, 30. Wessely and Schönol, *ibid.*, 1938, **71**, 10.

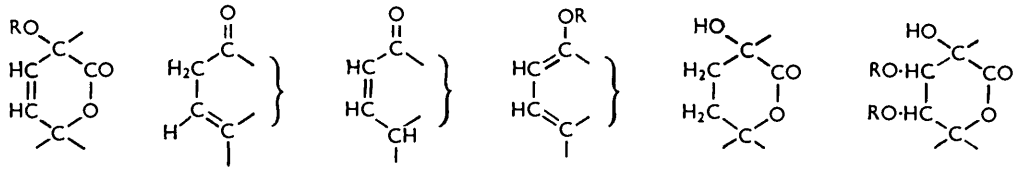
[2] Feist, Rintelen, and Kuntz, *Annalen*, 1935, **517**, 119; earlier work is cited also; Feist, Kuntz, and Brachvogel, *ibid.*, 1935, **519**, 124, **521**, 184; Feist and Brachvogel, *ibid.*, 1936, **522**, 185; Feist, Brachvogel, and Völksen, *ibid.*, 1936, **523**, 289; Feist and Völksen, *ibid.*, 1938, **534**, 41.

way during the hydrogenation. This eliminates a β-lactone formulation for lactone (*A*) which would, otherwise, explain the easy decarboxylation.

It seemed to us *ab origine* that these difficulties would be overcome if columbin were a βγ-unsaturated α-hydroxy-δ-lactone as in (I; R = H). Decarboxycolumbin would then be (II) or (III), whilst *iso*columbin acetate would be (I; R = Ac) and *O*-methyl*iso*columbin (I; R = Me).* Hydrogenation of the ethylenic linkage of (I) would then, of course, prevent the decarboxylation. Decarboxy*iso*columbin acetate and decarboxy-*O*-methyl-*iso*columbin would be represented by (IV; R = Ac and Me, respectively).

In agreement with these formulations columbin showed bands in the infrared region as follows: (in $CHCl_3$) at 1750 (γ- or δ-lactone), 1725 (δ-lactone), and 3620 (hydroxyl); (in Nujol) at 1745 (γ- or δ-lactone), 1705 (δ-lactone), and 3550 cm.$^{-1}$ (hydroxyl). In the ultraviolet region columbin absorbed only at 205 mμ (ε 6200; end absorption). Decarboxy*iso*columbin acetate and decarboxy-*O*-methyl*iso*columbin, on the other hand, absorbed like columbin and also at 272 mμ (ε 6800) and at 277 mμ (ε 5400), as would be expected for derivatives of (IV). Decarboxycolumbin itself showed in the ultraviolet region end absorption like that of columbin, and a distinct maximum at 285 mμ (ε 40; in dioxan). The spectral data show clearly that this compound is the βγ-unsaturated ketone (II), not the alternative (III); comparative infrared data strengthen these conclusions. Thus decarboxy-*O*-methyl*iso*columbin showed bands (in $CHCl_3$) at 1740 (γ- or δ-lactone), 1645 and 1585 (vinyl ether[3]), and 1150 cm.$^{-1}$ (ether). Decarboxycolumbin absorbed (in $CHCl_3$) at 1720 (γ- or δ-lactone) and 1710 (*cyclo*hexanone or aliphatic ketone), and (in Nujol) at 1708 cm.$^{-1}$ (superimposed lactone and ketone bands). There was no indication of contamination by an αβ-unsaturated ketonic function.

Conclusive chemical proof for the grouping (I; R = H) in columbin was secured as follows. Selective hydrogenation of columbin or *iso*columbin over palladised calcium



(I) (II) (III) (IV) (V) (VI)

carbonate afforded, respectively, dihydrocolumbin and dihydro*iso*columbin. Both compounds can be represented as (V) and, in agreement with this, were stable to heat. Mild alkali treatment of dihydrocolumbin gave, as expected, the dihydro*iso*columbin.

Treatment of *iso*columbin with osmium tetroxide in dioxan afforded *iso*columbindiol (VI; R = H).† In agreement with its formulation this compound was stable to heat, consumed two mols. of lead tetra-acetate, and showed an ultraviolet spectrum typical of a furan (see below). Whereas attempted acetylation of columbin or *iso*columbin with acetic anhydride–pyridine was without effect, mild acetylation of *iso*columbindiol with these reagents gave the diacetate (VI; R = Ac). One must conclude that the original hydroxyl group of columbin and of *iso*columbin is tertiary and that *iso*columbindiol contains two secondary hydroxyl groups, as already indicated in (VI; R = H) and preceding formulæ. In order to explain that hydrogenation of columbin and *iso*columbin gives compounds that do not titrate (under the same conditions as the precursors titrate) as lactones, we note that the α-hydroxy-δ-lactone ring in the hydrogenated products is less strained than in columbin or *iso*columbin and therefore less liable to rupture by

* We are assuming at this stage that the change from columbin to *iso*columbin involves a different part of the molecule from that concerned in the decarboxylation process. This is confirmed in the sequel.

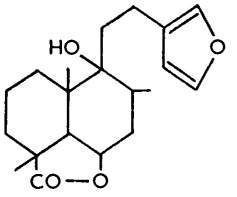
† Dr. W. Rigby kindly suggested the cleavage of osmates with ammonium polysulphide solution. We find that gaseous hydrogen sulphide, passed in to saturate the original solution, gives very satisfactory results and is a preferred method for working up such reactions. For details see Experimental.

[3] Meakins, *J.*, 1953, 4170.

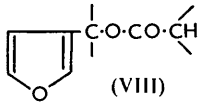
hydrolysis. In agreement with this, dihydrocolumbin titrated as a monolactone under mild alkaline conditions, but as a dilactone on more vigorous treatment with alkali.

In considering the analysis of columbin one must account for the equivalent of 10 double bonds. Accepting prior evidence [1,2] for a bicyclic carbon skeleton and noting the two lactone rings and associated ethylenic linkage, we have now to accommodate 3 double bonds. These double bonds are revealed only by end absorption in the ultraviolet region (see above). It seemed to us that the 3 double bonds and the residual uncharacterised oxygen atom of columbin were probably involved in a furan ring. In agreement with this view, both columbin and marrubiin (VII) [14] are very sensitive to chromic acid oxidation (see comparative rates given in the Experimental). Also the spectrum of dihydrocolumbin ($\lambda_{max.}$ 210 mμ; ε 5700) is superimposable on that of marrubiin.[4] The difference in spectrum between columbin (see above) and dihydrocolumbin is what would be expected if columbin contained both a furan ring and an ethylenic linkage. The infrared spectrum (Nujol) of columbin showed many bands in common with marrubiin at frequencies previously regarded as characteristic [4] for the furan ring.

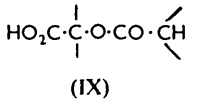
The furan nature of columbin was established as follows. Ozonolysis of dihydrocolumbin afforded a crystalline acid, $C_{17}H_{22}O_7$. When the water-soluble moiety of the ozonolysis product was methylated with diazomethane and chromatographed two methyl esters were obtained. The major product was the methyl ester of this acid $C_{17}H_{22}O_7$. The minor product, obtained always in variable yield, was the methyl ester of an acid, $C_{18}H_{22}O_8$. The presence of a ketone group was shown by the ultraviolet absorption spectrum. In contrast, the $C_{17}H_{22}O_7$ acid showed no absorption at all at 220—320 mμ and only insignificant absorption down to 205 mμ. The formation of these compounds is best explained if dihydrocolumbin is a β-substituted furan as in (VIII), the $C_{17}H_{22}O_7$ acid being (IX) and the $C_{18}H_{22}O_8$ keto-acid being (X). Ozonolysis of dihydrocolumbin also gave formic acid; there was no indication of any higher acid.



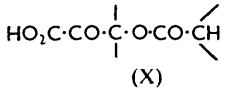
(VII)



(VIII)



(IX)



(X)

The easy hydrogenolysis [1,2] observed with columbin, decarboxycolumbin, and related compounds (see above) requires explanation. The alkyl-oxygen atom of lactone (*B*) (which, because of its reversible opening, cannot be vinylic) must be attached allylically with respect to some unsaturated substituent. Since hydrogenation of the βγ-unsaturated lactone system (*A*) *precedes* hydrogenolysis, it must be the furan ring, not the ethylenic linkage, which is responsible, as already implied in formulæ (VIII), (IX), and (X). In agreement the pK_a (in water) of the $C_{17}H_{22}O_7$ acid was 3·5, as expected somewhat stronger than that of lactic acid (3·9).[5] Clearly the $C_{17}H_{22}O_7$ acid has an electronegative α-substituent, as already allowed for in formula (IX).

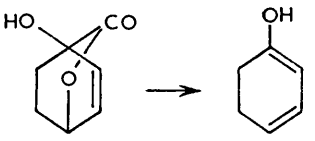
The nature of the conversion of columbin into *iso*columbin can now be discussed. The most reasonable explanation of this change is that it involves epimerisation α- to one of the lactonic carbonyl groups. Lactone (*A*) cannot be involved because decarboxycolumbin is a ketone, not an aldehyde (no aldehyde band in the infrared spectrum), and therefore there is no α-hydrogen in this lactone ring. We must conclude that it is the carbonyl group of lactone (*B*) which is responsible, as suggested in partial formulæ (VIII), (IX), and (X). There is nothing in the chemistry or spectral properties of *iso*columbin and its

[4] Cocker, Cross, Duff, Edward, and Holley, *J.*, 1953, 2540; Hardy and Rigby, *Chem. and Ind.*, 1953, 1150; and later papers from both Cocker and Rigby and their respective collaborators.
[5] Dietzel and Rosenbaum, *Z. Elektrochem.*, 1927, **33**, 196.

derivatives which contradicts such an interpretation. Thus, *iso*columbin showed the same ultraviolet absorption spectrum (ε 6500 at 205 mμ) as columbin, and in the infrared region ($CHCl_3$ solution) gave bands at 1748 (superimposed lactones) and 3520 cm.$^{-1}$ (hydroxyl).

The size of lactone ring (*A*) is defined by the work already discussed. The size of lactone ring (*B*) has not been established with certainty by the infrared spectrum method. Thus most of the data recorded above could be interpreted as indicating either a γ- or a δ-lactone ring. Examination of some dihydro-derivatives appeared to favour a γ-formulation. Thus dihydrocolumbin showed bands (in chloroform) at 1730 (δ-lactone) and 1750 (γ- or δ-lactone), but dihydro*iso*columbin absorbed at 1750 (two lactones; if one δ- then the other should be γ-). We believe that a final decision on this point must await the completion of degradational experiments now in hand.

The spectral data for decarboxy*iso*columbin acetate and decarboxy-*O*-methyl*iso*columbin recorded above are consistent only with the placing of the chromophore in *cisoid* conformation within a five- or six-membered ring. The infrared spectrum of decarboxycolumbin (see above) supports the latter alternative and this is confirmed by drastic degradational experiments to be discussed in Part II and by the infrared spectra of decarboxyoctahydrocolumbin. The latter compound showed (in bromoform) bands at 1720 (*cyclo*hexanone) and 1755 cm.$^{-1}$ (carboxyl). Although the transition state for a decarboxylation of the type now proposed [(XI) ⟶ (XII)] contravenes the Bredt rule it is, nevertheless, not without analogy.[6]



(XI) (XII)

With the nature of the functional groups of columbin established and having regard to earlier work on the drastic degradation of the molecule,[1,2] it is possible to propose several plausible structures. We defer such speculation until the completion of experiments now in hand.

Experimental

For general experimental directions see *J.*, 1952, 2339.

Infrared spectra were kindly determined by Glaxo Laboratories Limited. Ultraviolet absorption spectra were taken in ethanol (unless stated to the contrary), the Unicam S.P.500 Spectrophotometer being used. Unless stated to the contrary the light petroleum used was of b. p. 40—60°; $[\alpha]_D$ are recorded in pyridine solution unless stated otherwise. Columbin and its derivatives were prepared as detailed in the literature.[1,2] All compounds were characterised by m. p. and rotation; the physical constants agreed with those already recorded,[1,2] particularly with the data of Wessely *et al.*[1]

iso*Columbin Acetate.*—Columbin (109 mg.), anhydrous sodium acetate (440 mg.), and acetic anhydride (3 ml.) were refluxed for 5 hr. Crystallisation of the product from acetone–ethanol afforded *iso*columbin acetate, m. p. 225—226° (decomp.), $[\alpha]_D$ +28° (*c*, 1·03 in C_5H_5N). Wessely *et al.*[1a] recorded m. p. 230°, $[\alpha]_D$ +24° (in C_5H_5N). Columbin and *iso*columbin were unchanged after treatment with pyridine–acetic anhydride either overnight or on the steam-bath for 1 hr.

Chromic Acid Oxidation of iso*Columbin and Marrubiin.*—The compound (about 35 mg.) in " AnalaR " acetic acid (5 ml.) was treated at room temperature with a solution of chromic trioxide in the same solvent (0·6N; 5 ml.), and at intervals aliquot parts (1 ml.) were titrated in the usual way. The consumption of " oxygen " was as follows: marrubiin (kindly supplied by Dr. W. Rigby) 2·8 (5 min.), 4·4 (1 hr.), 4·8 (2 hr.), 5·4 (5 hr.); *iso*columbin 2·2 (5 min.), 3·2 (1 hr.), 4·0 (2 hr.), 5·4 (5 hr.).

Dihydrocolumbin.—Columbin (1·0 g.) in ethyl acetate (50 ml.) was hydrogenated over 1% palladised calcium carbonate (200 mg.) until one mol. of hydrogen had been absorbed (usually about 2 hr.). Trial experiments with a micro-hydrogenator had shown that under these conditions one mol. of hydrogen was absorbed relatively rapidly and a further three mol. (to give octahydrocolumbin) relatively slowly. The catalyst was removed by filtration and the solvent was evaporated *in vacuo*. Crystallisation from ethanol gave *dihydrocolumbin*

[6] Diels and Alder, *Annalen*, 1931, **490**, 257.

(50% or more depending on the purity of the starting material), m. p. 232—233°, $[\alpha]_D$ +5° (*c*, 3·48), $\lambda_{max.}$ 210 mμ (ε 5700) (Found: C, 66·5; H, 6·65. $C_{20}H_{24}O_6$ requires C, 66·65; H, 6·7%). Dihydrocolumbin (102·5 mg.) in absolute ethanol (8 ml.) was treated at room temperature for 16 hr. with aqueous sodium hydroxide (1N; 3 ml.) [Found: equiv., 355. $C_{20}H_{24}O_6$ requires equiv., 360 (one lactone group) or 180 (two lactone groups)]. Dihydrocolumbin (114·3 mg.), absolute ethanol (1 ml.), and aqueous sodium hydroxide (1N; 2 ml.) were heated on the steam-bath under nitrogen for 6 hr. (Found: equiv., 206). Finely divided dihydrocolumbin (128·8 mg.), absolute ethanol (1·0 ml.), and aqueous sodium hydroxide (1N; 2 ml.) were left at room temperature for 16 hr. (being occasionally shaken for the first hour) (Found: first equiv., 368). Aqueous sodium hydroxide (1N; 2 ml.) was added to the neutralised solution (phenolphthalein), which was then heated on the steam-bath for 6 hr. under nitrogen (Found: second equiv., 404).

Dihydroisocolumbin.—(a) *From dihydrocolumbin.* Dihydrocolumbin (1·0 g.) in ethanol (20 ml.) and aqueous potassium hydroxide (1N; 7·5 ml.) were heated on the steam-bath for 2 min. (clear solution). The solution was acidified (N-hydrochloric acid) and concentrated *in vacuo*. The precipitate was recrystallised from water containing a little ethanol, giving *dihydroisocolumbin* (long needles) (400 mg.), m. p. 233—235°, $[\alpha]_D$ +32° (*c*, 2·86) (Found: C, 66·5, 66·65; H, 6·4, 6·4. $C_{20}H_{24}O_6$ requires C, 66·65; H, 6·7%).

(b) *From* isocolumbin. *iso*Columbin (1·0 g.) was hydrogenated as for columbin (see above). Crystallisation from water containing a little ethanol afforded dihydro*iso*columbin, m. p. 233—235°, undepressed on admixture with material prepared as under (a).

Ozonolysis of Dihydrocolumbin.—(*a*) Dihydrocolumbin (1·0 g.) in chloroform (30 ml.) was treated with ozone at 0° until the furan spectrum had disappeared. Water (6 ml.) was added and the mixture refluxed for 10 min. The chloroform layer was removed and the aqueous layer was evaporated to dryness under reduced pressure. The residue (200 mg.) was crystallised from water to give *dihydrotrisnorcolumbinic acid*, m. p. 118° (decomp.), $[\alpha]_D$ −5° (*c*, 1·78), $\varepsilon < 400$ at 209 mμ, $\varepsilon = 0$ at 280 mμ, pK_a 3·55 (hydrated), 3·45 (anhydrous) (Found, in material dried for 12 hr. at 80° *in vacuo*: C, 55·45, 55·5; H, 6·85, 6·65. $C_{17}H_{22}O_7,1\frac{1}{2}H_2O$ requires C, 55·9; H, 6·9%). The acid (25·23 mg.) was dried at 120° *in vacuo* to constant weight (42 hr.); the loss in weight (2·1 mg.) is equivalent to 1·52 mol. of water. The dried *acid* had m. p.—234—237°, $[\alpha]_D$ −6° (*c*, 2·56) (Found: C, 59·55, 59·5; H, 6·4, 6·5. $C_{17}H_{22}O_7$ requires C, 60·3; H, 6·55%). The acid was treated in acetone solution with ethereal diazomethane. Crystallisation from benzene afforded *methyl dihydrotrisnorcolumbate*, m. p. 200—202°, $[\alpha]_D$ −12° (*c*, 2·30) (Found: C, 61·2; H, 6·6. $C_{18}H_{24}O_7$ requires C, 61·35; H, 6·85%).

(*b*) Dihydrocolumbin (1·0 g.) was treated with ozone and then processed exactly as described above. The residue from evaporation of the aqueous layer was dissolved in acetone and treated with an excess of ethereal diazomethane. The product was chromatographed over silica gel (15 g.) in 1 : 9 acetone : benzene (16—20 fractions). The main product, eluted in the earlier fractions, was the ester, m. p. 200—202°, reported above. The minor product, eluted in the last few fractions, gave *methyl dihydro-oxobisnorcolumbate*, m. p. 247—250° (from benzene), $[\alpha]_D$ −10° (*c*, 1·13), $\lambda_{max.}$ 285 mμ [ε 30 (in dry dioxan)] (Found: C, 59·75; H, 6·5. $C_{19}H_{24}O_8$ requires C, 60·0; H, 6·35%).

(*c*) Dihydrocolumbin (1·0 g.) was treated with ozone as described above except that the aqueous layer was distilled and the distillate examined for volatile acids. The distillate, titrated with standard potassium hydroxide solution (phenolphthalein), contained 0·6 mol. of acid. This was converted into the *p*-bromophenacyl ester, and the product chromatographed over neutralised alumina. Elution with benzene gave only one ester (from light petroleum) identified by m. p., mixed m. p., and crystal form as *p*-bromophenacyl formate.

Treatment of iso*Columbin with Osmium Tetroxide.*—Osmium tetroxide (170 mg.) in dry purified dioxan (5 ml.) was added to *iso*columbin (170 mg.) in the same solvent (5 ml.). The solution was left at room temperature for 48 hr. and then saturated with hydrogen sulphide. The black precipitate was filtered off and the dioxan solution evaporated to dryness under reduced pressure. Crystallisation from ethanol furnished *isocolumbindiol* (90%), m. p. 261—262° $[\alpha]_D$ +34° (*c*, 1·49), $\lambda_{max.}$ 210 mμ (ε 5500) (Found: C, 61·0; H, 6·4. $C_{20}H_{24}O_8$ requires C, 61·2; H, 6·15%). This diol (11·5 mg.) in " AnalaR " acetic acid (2 ml.) was treated with lead tetra-acetate (saturated solution in " AnalaR " acetic acid; 8 ml.). After 15 min. the uptake of lead tetra-acetate was 1·6 mols., after 6 hours 2·1 mols.

*iso*Columbindiol (320 mg.) in dry pyridine (5 ml.) and acetic anhydride (2·5 ml.) were left overnight at room temperature. Crystallisation of the product from ethyl acetate–light petroleum (b. p. 60—80°) gave the *diacetate*, m. p. 233—235°, $[\alpha]_D$ +51° (*c*, 2·42) [Found:

C, 60·45, 60·75; H, 6·0, 6·2; Ac, 19·5 (preliminary alkaline hydrolysis). $C_{24}H_{28}O_{10}$ requires C, 60·5; H, 5·9; Ac, 18·1%].

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