940. *Diterpenoid Bitter Principles. Part V.* The Constitution of Palmarin and its Congeners.*

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The presence of 1,2-epoxide groups in βγ-relationship to the " A lactone " of palmarin and isojateorin has been demonstrated. Isojateorin and dihydroisocolumbin have been converted into a common derivative, thus proving that isojateorin is the 1,2-epoxide of isocolumbin. The constitutions of all the Colombo-root bitter principles have, therefore, now been determined. The application of nuclear magnetic resonance measurements to the constitutional and stereochemical problems in these compounds has been described.

Colombo root contains three bitter principles, columbin [1] (I), chasmanthin, and jateorin.[2] The last two are epimers about C-12 in the partial formulation (II). For convenience of isolation,[2] constitutional investigations must be carried out with palmarin and isojateorin. These compounds are formed from chasmanthin and jateorin by alkali-induced epimerisation at C-8 and can, therefore, also be represented by (II).[2]

Palmarin, isojateorin, and their epimers differ by one oxygen atom from columbin. Since a biogenetic relationship appeared very probable we adopted already in 1955 the hypothesis that palmarin was the epoxide (IIIa) of isocolumbin, a compound formed from columbin by epimerisation at C-8. Earlier investigations seemed, however, to disprove this simple relationship. The presence of an oxiran ring could not be demonstrated by the conventional tests of sensitivity to acids and cleavage by lithium aluminium hydride. However, nuclear magnetic resonance measurements, which are discussed in detail below, caused us to examine again our original hypothesis which, in the event, is true. For convenience of exposition we shall use the correct formulæ throughout the present paper.

In our earlier work [2] we reduced palmarin (IIIb) with lithium aluminium hydride to the hemiacetal (IVb) which on periodate cleavage and mild alkaline hydrolysis afforded the trihydroxy-ketone (Vb). Treatment of the latter product with toluene-*p*-sulphonyl chloride and pyridine furnished an anhydro-monotoluene-*p*-sulphonate (VIb). This is no doubt produced by solvolysis of an initially formed ditoluene-*p*-sulphonate, giving an incipient carbonium ion α to the furan ring, which then cyclises intramolecularly with a neighbouring hydroxyl group. The presence of the αβ-epoxy-ketone group in compound (VIb) was demonstrated [3] by reduction with chromous chloride to the αβ-unsaturated ketone (VIIb). By starting with isojateorin (IIIa) a similar toluene-*p*-sulphonate (VIa) was prepared and reduced to the corresponding αβ-unsaturated ketone (VIIa).

The formation of these unsaturated ketones effectively solves the constitutional problem. However, we were concerned that the carbonyl frequency of the " A lactone " in palmarin and its derivatives should be such as to indicate γ- rather than δ-lactonisation. There are, of course, special strain factors in the 2,3-epoxy-1,4-lactones (boat conformation) of palmarin (IIIb) and its derivatives which might well exalt the lactone frequency abnormally. Additional proof of the points of attachment of the " A lactone " was secured by direct correlation with columbin (I).

Reduction of isocolumbin with lithium aluminium hydride and further processing as for palmarin was expected to furnish the unsaturated ketone (VII) of either the palmarin (VIIb) or the isojateorin (VIIa) series. In the event, the product, whilst it could not be completely purified, corresponded to (VIIa) rather than (VIIb). The reaction sequence was then repeated with dihydroisocolumbin to furnish the ketone (VIIIa). The same

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* Part IV, preceding paper.

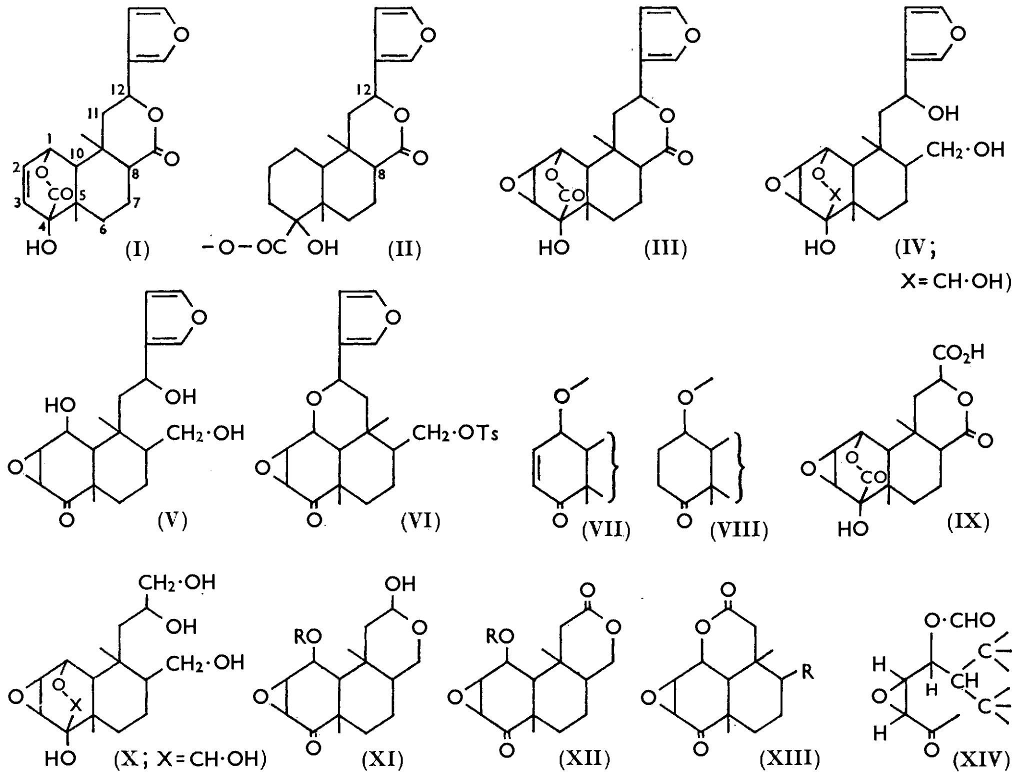
[1] Barton and Elad, *J.*, 1956, 2085, 2090.

[2] Barton, Overton, and Wylie, *J.*, 1962, preceding paper.

Cole and Julian, *J. Org. Chem.*, 1954, **19**, 131.

compound was obtained by hydrogenation of the unsaturated ketone (VIIa) from isojateorin. The proof of the constitutions of all the Colombo-root bitter principles is thus complete.

We can now assign formulæ to all the compounds which were only partly formulated in the preceding paper.[2] Trisnorpalmarinic acid must have structure (IXb; R = H), its lithium aluminium hydride reduction product (Xb), the periodate cleavage product of the latter (XI; R = CHO), and the derived lactone (XII; R = CHO). The saponification product of the last must be represented as (XIII; R = CH_2·OH), and the derived acid as (XIII; R = CO_2H). Amplification of other formulæ given in the preceding paper[2] will be obvious.



a = The isocolumbin–isojateorin series.
b = The palmarin series (epimeric at C-12).

As mentioned above, the nature and orientations of substituents in ring A of palmarin could be derived by the application of nuclear magnetic resonance spectroscopy. The key compound in this investigation was the keto-formate (XII; R = CHO), the spectrum of which is shown in the Figure. The complete analysis of this spectrum involved extensive use of spin-decoupling experiments in which the single side-band technique developed by Turner[4] was used. Certain absorptions can be assigned on the basis of known features of the molecule. Thus, in addition to the two quaternary methyl groups, there is an AB quartet (τ_A 6·83; τ_B 7·86; J_{AB} 16·8 c./sec.) associated with the protons at position 11, and an ABX system [AB region near 5·7; X band near 7·6 (diffuse)] arising from the 8-proton and the adjacent methylene group of the modified " B lactone." The starting point for the analysis of the remainder of the spectrum is the absorption of the " aldehydic "

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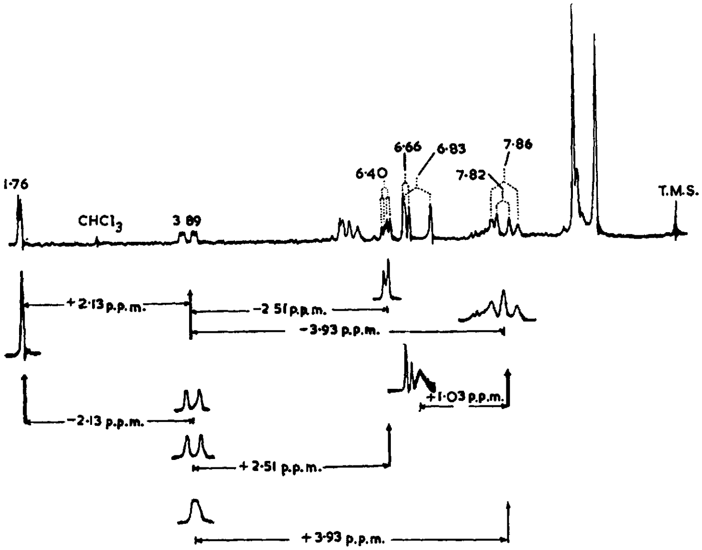
[4] Turner, *J.*, 1962, 847.

proton of the formate group which is observed as a doublet ($J \sim 1·0$ c./sec.) at 1·76. This proton must be coupled, *via* four σ-bonds, with a proton on the carbon atom bearing the ester group.* Spin-decoupling located the absorption of this proton as a multiplet at 3·89 with a fine structure suggesting coupling with three different protons ($J = 8·9$, 2·3, *ca.* 1·0 c./sec.). The large coupling (8·9 c./sec.) could be shown by decoupling (see Figure) to arise from a proton absorbing as a doublet at 7·82. Thus, this doublet was simplified to a singlet when recorded with simultaneous strong irradiation of the proton absorbing at 3·89. Absorption at 7·82 suggests a methine proton, and since the band is a doublet it is probable that the remaining two carbon atoms to which it is attached do not carry protons.† The proton absorbing at 3·89 is further coupled (J 2·3 c./sec.) to a third proton which was shown by spin-decoupling to give rise to the double doublet (J 2·3, 4·7 c./sec.) at 6·40. The proton absorbing at 6·40 is in turn seen to be coupled (J 4·7 c./sec.) to one absorbing at 6·66. This could not be proved by decoupling as the available experimental method requires that the strong and the weak radiofrequency fields differ in frequency by at least 40 c./sec. However, the doublet at 6·66 could not be collapsed by irradiation with frequencies greater than 40 c./sec.; further, the spacings and intensity patterns of the bands at 6·40 and 6·66 leave no doubt that they arise from mutually interacting protons. This is particularly clear if the comparison is made between the 6·40 band in the spectrum in which the proton at 3·89 is irradiated, and the 6·66 band from which the lowest-field component of the AB quartet from the 11-methylene protons has been removed by irradiation of the B-proton (see Figure). The absorption at 6·40 and 6·66 must arise from protons attached to carbon atoms carrying singly bonded oxygen, and, in fact, these carbon atoms must be the points of attachment of the ether-oxygen. Thus, palmarin contains a 1,2-



Nuclear magnetic single and double resonance spectra of the keto-formate (XII; R = CHO).

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* Spin–spin interactions across four σ-bonds have been observed in a number of systems [5, 6] and, in several of these, aldehydic protons are involved.

† The possibility that one or both of these carbon atoms carry a proton which makes a dihedra angle of *ca.* 90° with the methine proton, thus causing the coupling constant to vanish, cannot be rigorously excluded.

[5] Davis, Lutz, and Roberts, *J. Amer. Chem. Soc.*, 1961, **83**, 247.

[6] Schneidegger, Schaffner, and Jeger, *Helv. Chim. Acta*, 1962, **45**, 400.

epoxide (oxiran ring). Generally,[7] protons of 1,2-epoxides (oxirans) absorb in the range 7·0—7·8 but both the α- and the β-proton of αβ-epoxy-ketones absorb in the range 6·2—6·8. Thus, the partial structure (XIV) can be written for the keto-formate, and this formulation is consistent with the observation that the 6·66 band is a simple doublet. Clearly, the αβ-epoxy-γ-formyloxy-ketone can only be accommodated in ring A as in structure (XII; R = CHO). The remaining four protons in ring B were located, by integration, under the methyl absorption at 8·68. Nuclear magnetic resonance spectra also provided confirmation of the structures of the αβ-unsaturated ketones (VIIa and b). Pairs of doublets (*J* 10·2 c./sec.) are observed at 4·11 and 3·32, and at 4·10 and 3·08, in the spectra of the unsaturated ketones derived from isojateorin and palmarin, respectively. These doublets exhibit additional fine splitting arising from weak coupling with the γ-proton. In each case, the high- and the low-field doublets are assigned to the α- and the β-protons, respectively, their positions being in good agreement with those of the corresponding protons of cyclohex-2-enone [4·00, 3·10 (*J* 10·2 c./sec.)].

Examination of the spectra of the toluene-*p*-sulphonates (VIa and b), and several decoupling experiments, allowed assignments to be made to the absorption of the proton at the 12-position in each isomer. In the isojateorin series, this absorption was a triplet at 5·33 with an overall width of 17·0 c./sec. The protons of the adjacent methylene group are accidentally equivalent and give rise to a doublet at 8·21. The value of 17·0 c./sec. is the sum of the coupling constants of the 12-proton and the two 11-protons, and is in good agreement with the values expected for diaxial (*ca.* 13 c./sec.) and axial–equatorial (3·0—7·0 c./sec.) interactions in a normal chair conformation.[8] The absorption of the 12-proton in the palmarin derivative (VIb) is a double doublet at 5·11. The width of this band is 13·5 c./sec., which is probably too large for the sum of the diequatorial and axial–equatorial coupling constants. However, in the chair conformation an axial furan ring would suffer two serious 1,3-diaxial non-bonding interactions and it is possible that the six-membered heterocyclic ring might prefer a distorted boat conformation in which the furan ring is quasi-equatorial. We do not consider the above evidence is sufficiently definitive to be accepted as a proof of stereochemistry. The investigations on the stereochemistry of columbin reported by Overton, Weir, and Wylie [9] have direct application to this problem. It is our understanding that Dr. K. H. Overton will discuss the matter fully in a later and definitive paper on the stereochemistry of all the Colombo-root bitter principles.

Owing to an unfortunate oversight, one paragraph was omitted from the experimental section of an earlier paper in this series [10] on the chemistry of clerodin. We have added the missing paragraph to the Experimental section of the present paper.

EXPERIMENTAL

M. p.s were taken on the Kofler block. Ultraviolet absorption spectra were determined for ethanol, $[\alpha]_D$ for chloroform, and infrared spectra for Nujol mulls. Grade III alumina was used for chromatography. Light petroleum refers to the fraction of b. p. 40—60°.

Nuclear magnetic resonance spectra were measured with a Varian 4300 spectrometer operating at 56·4 Mc./sec. Deuterochloroform solutions were used throughout and the spectra were calibrated by the side-band technique with a Muirhead-Wigan decade oscillator, model D-695-A.

Reduction of the Toluene-p-*sulphonate* (VIb) *from Palmarin.*—The reducing agent was prepared in the following way.[11] Zinc dust (7·0 g.) was amalgamated with mercuric chloride (2·6 g.) in water (10 ml.) containing concentrated hydrochloric acid (several drops). After thorough washing, the amalgam was treated with chromic chloride hydrate (2·7 g.) in N-hydrochloric acid (10 ml.) until reduction was complete. The solution was transferred by nitrogen

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[7] L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, p. 55.

[8] Karplus, *J. Phys. Chem.*, 1960, **64**, 1793.

[9] Overton, Weir, and Wylie, *Proc. Chem. Soc.*, 1961, 211.

[10] Barton, Cheung, Cross, Jackman, and Martin-Smith, *J.*, 1961, 5061.

[11] See Hatfield, *Inorg. Syntheses*, 1950, **3**, 148.

pressure through a glass filter under nitrogen into the toluene-*p*-sulphonate (VIb) (66 mg.) in acetone (5 ml.) and left overnight under nitrogen. The acetone was removed *in vacuo* and the solution extracted with chloroform. The product was chromatographed over alumina (1·9 g.). Elution with benzene gave the desired unsaturated *ketone* (VIIb) (52 mg.). Recrystallised from chloroform–pentane this had m. p. 145—147°, $[\alpha]_D$ −30° (*c* 1·23), $\lambda_{max.}$ 222 mμ (ε 20,000), $\nu_{max.}$ 1680 (cyclohexenone) and 1605 (toluene-*p*-sulphonate) cm.$^{-1}$ (Found: C, 66·5; H, 6·55. $C_{26}H_{30}O_6S$ requires C, 66·35; H, 6·45%).

*Reduction of the Toluene-*p*-sulphonate* (VIa) *from Isojateorin.*—Isojateorin was reduced with lithium aluminium hydride and further processed as with palmarin to the toluene-*p*-sulphonate stage (1·15 g. of mixed toluene-*p*-sulphonates from 3·05 g. of isojateorin). Chromatography over alumina gave, on elution with benzene–light petroleum mixtures, first non-crystalline material (130 mg.), then the toluene-*p*-sulphonate (VIb) from palmarin (150 mg.; identified by m. p., mixed m. p., and $[\alpha]_D$) followed by mixed toluene-*p*-sulphonates (290 mg.), and finally the desired *toluene-*p*-sulphonate* (VIIa) (500 mg.) from isojateorin. Recrystallised from chloroform–pentane this had m. p. 118—120°, $[\alpha]_D$ +41° (*c* 1·34), $\lambda_{max.}$ 222 mμ (ε 15,400), $\nu_{max.}$ 1710 (cyclohexanone) and 1603 (toluene-*p*-sulphonate) cm.$^{-1}$ (Found: C, 64·1; H, 6·4. $C_{26}H_{30}O_7S$ requires C, 64·2; H, 6·2%). This compound (66 mg.) was reduced with chromous chloride as described above for the palmarin series. The product was chromatographed over alumina. Elution with benzene–light petroleum (3 : 2) gave the unsaturated *ketone* (VIIa) of the isojateorin series. Recrystallised from ether–pentane this had m. p. 105—108°, $[\alpha]_D$ +18° (*c* 1·02), $\lambda_{max.}$ 222 mμ (ε 20,200), $\nu_{max.}$ 1680 (cyclohexenone) and 1603 (toluene-*p*-sulphonate) cm.$^{-1}$ (Found: C, 66·6; H, 6·3. $C_{26}H_{30}O_6S$ requires C, 66·35; H, 6·45%).

The above unsaturated ketone (VIIa) (78 mg.) was hydrogenated in ethyl acetate (7 ml.) over 1% palladised calcium carbonate (70 mg.) for 12 hr. (1 mol. uptake). Chromatography of the product over alumina gave, on elution with benzene, the *ketone toluene-*p*-sulphonate* (VIIIa) (50 mg.). Recrystallised from ether–pentane this had m. p. 112—114°, $[\alpha]_D$ +95° (*c* 0·50), $\lambda_{max.}$ 222 mμ (ε 13,200), $\nu_{max.}$ 1705 (cyclohexanone) and 1603 (toluene-*p*-sulphonate) cm.$^{-1}$ (Found: C, 66·2; H, 6·8. $C_{26}H_{32}O_6S$ requires C, 66·1; H, 6·85%).

Experiments in the Isocolumbin Series.—(*a*) When isocolumbin was reduced with lithium aluminium hydride and further processed as for palmarin [2] it gave a product which could not be completely purified even on careful chromatography. The infrared spectrum was essentially identical with that of the unsaturated ketone (VIIa) from isojateorin (m. p. not depressed) and different from that of the unsaturated ketone (VIIb) from palmarin.

(*b*) The dihydro-derivative [1] was reduced and further processed as for palmarin.[2] The crude product (2·0 g.; from 2·5 g. of dihydroisocolumbin) was chromatographed over alumina. Elution with benzene furnished the toluene-*p*-sulphonate (VIIIa) (700 mg.) from isojateorin {m. p., mixed m. p., $[\alpha]_D$ +98° (*c* 0·82) and ultraviolet and infrared spectra}.

Addition to J., 1961, 5072, *after line* 13 *from the Bottom.*—The diketone (XII; R = Me) (formula numbering as in the paper cited) (64 mg.) in methanol (3 ml.) was refluxed with sodium hypochlorite solution (0·25 ml., 0·70 equiv. of oxidant) for 15 min. Separation of the acidic fraction, and crystallisation from ether–light petroleum (b. p. 40—60°) gave the *keto-acid* (XXII; R = OH) as prisms (14 mg.), m. p. 210—222° (decomp.), $[\alpha]_D$ +4° (*c* 0·51), $\nu_{max.}$ (in Nujol) 2700—3500 (OH), 1725 (CO_2H) and 1705 (C=O) cm.$^{-1}$ (Found: C, 67·9; H, 8·2. $C_{19}H_{28}O_5$ requires C, 67·85; H, 8·4%).

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