273. *Natural Occurrence of Enantiomorphous Leucoanthocyanidins: (+)-Mollisacacidin (Gleditsin) and Quebracho (−)-Leucofisetinidin.*

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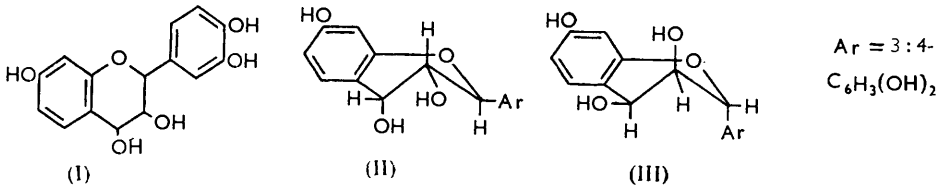
The identity of gleditsin with mollisacacidin is confirmed and this dextrorotatory form of 7 : 3′ : 4′-trihydroxyflavan-3 : 4-diol is shown to be enantiomorphous with the (−)-leucofisetinidin from quebracho wood. Formation of *iso*propylidene derivatives from the leucofisetinidins indicates that they are *cis*-3 : 4-diols, and hydrogenation suggests that the 2-aryl and the 3-hydroxyl group are in *trans*-relation.

Few leucoanthocyanidins have been isolated from natural sources since King and Bottomley showed that the flavan-3 : 4-diol melacacidin [1] from *Acacia melanoxylon* (Australian blackwood) has leucoanthocyanidin properties. A flavan-3 : 4-diol structure [2]

[1] King and Bottomley, *Chem. and Ind.*, 1953, 1368; *J.*, 1954, 1399.
[2] Chan, Forsyth, and Hassall, *Chem. and Ind.*, 1957, 264; Forsyth, Hassall, and Roberts, *ibid.*, 1958, 656.

has been established for peltogynol but, apart from cacao-leucocyanidin,[3] leucodelphinidin,[4] and the flavandiols recently reported by Seshadri (see below), the only additions to the leucoanthocyanidin series are mollisacacidin [5] from *Acacia mollissima* (black wattle), gleditsin [6,7] from *Gleditsia* (*Gleditschia*) *japonica*, and the (—)-leucofisetinidin [8] from *Schinopsis quebracho-colorado*. These three compounds have the 7 : 3′ : 4′-trihydroxyflavan-3 : 4-diol structure (I), and the identity of the two dextrorotatory forms (mollisacacidin and gleditsin) [7] is now confirmed by comparing the trimethyl ethers, the trimethyl ether diacetates, and the trimethyl ether *iso*propylidene derivatives (m. p.s, mixed m. p.s, and absorption spectra).



(I) (II) (III)

(—)-Leucofisetinidin,[8] isolated from quebracho wood, resembled mollisacacidin closely, except in the sign of rotation, and the enantiomorphous nature of the two leucoanthocyanidins and their derivatives was established by their infrared absorption curves and by formation of racemates from equal quantities of (+)- and (—)-compounds. Moreover the melting points of the racemates were not depressed [9] by admixture with inactive derivatives prepared from natural fustin [10] or from synthetic *trans*-dihydro-7 : 3′ : 4′-trimethoxyflavonol.[11]

The enantiomorphism of (+)-mollisacacidin (gleditsin) and the (—)-leucofisetinidin from quebracho wood is thus unequivocally established, and these compounds are the first examples of enantiomorphous forms of a flavonoid in Nature. Further, either mollisacacidin (gleditsin) or the enantiomorphous (—)-leucofisetinidin must have the (2*S*)-configuration [12] and thus constitute an exception among optically active flavonoids which, hitherto, have been found to occur with the (2*R*)-configuration.[13,14] The stereochemistry of these leucofisetinidins can now be inferred, except for their absolute configurations. The formation of *iso*propylidene derivatives of these compounds is regarded as strong evidence for the *cis*-configuration of the 3 : 4-diol grouping, and supports the inference drawn from the formation of an acidic borate complex.[5] Moreover fustin, which yields racemic leucofisetinidin on hydrogenation,[10] is almost certainly a *trans*-compound like dihydroquercetin [14] and other naturally occurring dihydroflavonols.[15] Mollisacacidin (gleditsin) and quebracho (—)-leucofisetinidin may therefore be assigned the 2 : 3-*trans*-3 : 4-*cis*-configuration for which two half-chair conformations (II) and (III) are possible (also their mirror images). Assignment of the 2 : 3-*trans*-3 : 4-*cis*-configuration to the leucofisetinidins is supported by the work of Chandorkar and Kulkarni [11] who have prepared (±)-7 : 3′ : 4′-trimethoxyflavan-3 : 4-diol and its diacetate by reduction of synthetic *trans*-(±)-7 : 3′ : 4′-trimethoxydihydroflavonol with lithium aluminium hydride. The identity of these compounds with our racemates has been established by mixed m. p. determinations, and by

[3] Forsyth and Roberts, *Chem. and Ind.*, 1958, 755.
[4] Hathway, *Biochem. J.*, 1958, **70**, 34.
[5] Keppler, *Chem. and Ind.*, 1956, 380; *J.*, 1957, 2721.
[6] Mitsuno and Yoshizaki, *J. Pharm. Soc. Japan*, 1957, **77**, 557, 1208.
[7] Clark-Lewis and Mitsuno, *J.*, 1958, 1724.
[8] Roux, *Chem. and Ind.*, 1958, 161.
[9] Clark-Lewis and Roux, *ibid.*, p. 1475.
[10] Roux and Freudenberg, *Annalen*, 1958, **613**, 56.
[11] Chandorkar and Kulkarni, *Current Sci.*, 1957, **26**, 354.
[12] Cahn, Ingold, and Prelog, *Experientia*, 1956, **12**, 81.
[13] Birch, Clark-Lewis, and Robertson, *J.*, 1957, 3586; see, however, Brown and Somerfield, *Proc. Chem. Soc.*, 1958, 236.
[14] Clark-Lewis and Korytnyk, *J.*, 1958, 2367.
[15] Mahesh and Seshadri, *Proc. Indian Acad. Sci.*, 1955, **41**, *A*, 210; Whalley, " Chemistry of Vegetable Tannins," Soc. Leather Trades' Chemists, Croydon, 1956, p. 151.

infrared measurements on the diacetate. The closely related melacacidin (7 : 8 : 3′ : 4′-tetrahydroxyflavan-3 : 4-diol) probably has the 2 : 3-*cis*-3 : 4-*cis*-arrangement of substituents:[16] melacacidin resembles *epi*catechin in having the 2 : 3-*cis*-configuration and the leucofisetinidins resemble catechin in the 2 : 3-*trans*-configuration.

Robinson and Robinson[17] classified the leucoanthocyanidins into three groups according to solubilities; these probably correspond to (*a*) polymeric leucoanthocyanidins, *e.g.*, polymeric flavan-3 : 4-diols formed by condensation analogous to that proposed for catechins,[18] (*b*) leucoanthocyanidin glycosides (*i.e.*, leucoanthocyanins), and (*c*) monomeric leucoanthocyanidins, *e.g.*, flavan-3 : 4-diols. Progress so far has been restricted to group (*c*) and it is interesting that the monomeric flavan-3 : 4-diols first isolated all possessed hydroxyl groups in the 7- and the 4′-, but not the 5-position. Seshadri and his co-workers recently[19] isolated and characterised a leucopelargonidin, two leucocyanidins, and two leucodelphinidins from natural sources. Few details are yet available, but both leucodelphinidins evidently differ from the material described by Hathway.[4]

Experimental

Infrared absorptions were recorded with a Grubb–Parsons S4 spectrometer (for mulls and solutions) and with a Perkin–Elmer Infracord (for potassium bromide discs). $C_2H_2Cl_4$ below refers to 1 : 1 : 2 : 2-tetrachloroethane.

Materials.—Mollisacacidin obtained from *Acacia mollissima* heartwood and (−)-leucofisetinidin from *Schinopsis quebracho-colorado* sapwood were purified by preparative paper chromatography on thick sheets as already described.[20] The penta-acetates and the 7 : 3′ : 4′-trimethyl ethers and their diacetates were prepared as described for the (−)-leucofisetinidin derivatives.[20] Gleditsin and its derivatives were presented by Dr. M. Mitsuno. For mollisacacidin Keppler [5] reports $[\alpha]_D^{18}$ +12·6° (1% in MeOH) and we found $[\alpha]_D^{12}$ +31·4° (0·8% in 1 : 1 $COMe_2$–H_2O). For gleditsin we found $[\alpha]_D^{16}$ +14° (0·5% in MeOH) and Mitsuno and Yoshizaki [6] report $[\alpha]_D^{12}$ +33·6° (in 1 : 1 $COMe_2$–H_2O).

Mollisacacidin Trimethyl Ether.[5]—Crystallisation from aqueous ethanol gave the *sesquihydrate* in needles, sintering at 76—77° (cf. ref. 5), m. p. 91—94°, and 129° after resolidification, $[\alpha]_D^{16}$ −10·3° (1·12% in $C_2H_2Cl_4$) (Found: C, 59·9; H, 6·6; OMe, 26·4; loss on drying for 6 hr. at 70° *in vacuo*, 7·4. $C_{18}H_{20}O_6$,1·5H_2O requires C, 60·2; H, 6·45; OMe, 25·9; 1·5H_2O, 7·5%). Material dried for 5½ hr. at 60° *in vacuo* apparently lost only one molecule of water of crystallisation (Found: loss, 4·7. $C_{18}H_{20}O_6$,1·5H_2O requires 1H_2O, 5·0%).

Mollisacacidin trimethyl ether diacetate crystallised from ethanol in needles, m. p. 102°, $[\alpha]_D^{16}$ −17·1° (1·52% in $C_2H_2Cl_4$) (Found: C, 63·0; H, 5·8; OMe, 22·4; Ac, 19·8. $C_{22}H_{24}O_8$ requires C, 63·45; H, 5·8; OMe, 22·4; Ac, 20·7%). For the gleditsin derivative, Dr. Mitsuno [21] reports $[\alpha]_D^{10}$ −19·4° (2·8% in pyridine) and m. p. 100·5—101·5°.

Mollisacacidin 7 : 3′ : 4′-Trimethyl Ether iso*Propylidene Derivative.*—Mollisacacidin trimethyl ether was treated with acetone as described for (±)-melacacidin tetramethyl ether [16] and gave the iso*propylidene derivative* which crystallised from ethanol in prisms, m. p. 120—122°, $[\alpha]_D^{12}$ +5·8° (1·03% in $C_2H_2Cl_4$) (Found: C, 67·7; H, 6·55. $C_{21}H_{24}O_6$ requires C, 67·7; H, 6·50%). For the gleditsin derivative we found m. p. 117—118°.

(−)- *and* (±)-*Leucofisetinidin 7 : 3′ : 4′-Trimethyl Ether* iso*Propylidene Derivative.*—Quebracho (−)-leucofisetinidin trimethyl ether {m. p.s 81—84°, 134° (lit.,[22] 125—127°), $[\alpha]_D^{16}$ +9·4° (1·12% in $C_2H_2Cl_4$) (lit.,[22] $[\alpha]_D^{25}$ −3·8° (2% in $C_2H_2Cl_4$)} was converted into its iso*propylidene derivative* by the same method; [16] this crystallised from ethanol in prisms, m. p. 120—122°, $[\alpha]_D^{12}$ −6·3° (1·04% in $C_2H_2Cl_4$) (Found: C, 68·0; H, 6·4%).

[16] King and Clark-Lewis, *J.*, 1955, 3384.
[17] Robinson and Robinson, *J.*, 1935, 744.
[18] Freudenberg and Alonso, *Annalen*, 1958, **612**, 78.
[19] Seshadri and Laumas, *J. Sci. Ind. Res., India*, 1958, **17**, *B*, 44, 167; Seshadri and Ganguly, *ibid.*, p. 168.
[20] Roux and Evelyn, *Biochem. J.*, 1958, **70**, 334.
[21] Personal communication from Dr. Mitsuno.
[22] Freudenberg and Weinges, *Annalen*, 1958, **613**, 61.

7 : 3′ : 4′-Trimethoxyflavan-3 : 4-diol, $[\alpha]_D^{15}$ −1·2° (0·82% in $C_2H_2Cl_4$), prepared by hydrogenation of fustin and subsequent methylation,[10] was similarly converted into the (±)-*isopropylidene derivative*, m. p. 134° (Found: C, 67·5; H, 6·5%). The product possessed slight optical activity, $[\alpha]_D^{15}$ −1·1° (0·94% in $C_2H_2Cl_4$).

Comparison of Mollisacacidin, Gleditsin, and (−)-Leucofisetinidin and their Derivatives.—(a) *The 7 : 3′ : 4′-trihydroxyflavan-3 : 4-diols.* Mollisacacidin, gleditsin, and quebracho (−)-leucofisetinidin all crystallised as dihydrates, in needles which melt with decomposition over a range. Mollisacacidin and gleditsin melted within the range 125—130°, alone or when mixed, when examined with a Leitz hot-stage microscope. A mixture of gleditsin and (−)-leucofisetinidin melted indefinitely in the range 127—146°.

Infrared spectra of mollisacacidin, gleditsin, and (−)-leucofisetinidin (Nujol mulls) were identical throughout the recorded range (2—15 μ). Mollisacacidin and (−)-leucofisetinidin (in KBr) gave identical curves which differed from that of the (±)-compound (prepared from fustin) as expected, although the curves were generally similar.

Paper chromatograms were sprayed with ammoniacal silver nitrate or 5% ethanolic toluene-*p*-sulphonic acid. The following R_F values were obtained for mollisacacidin, gleditsin, and (−)-leucofisetinidin, respectively: in BuOH–AcOH–H_2O (5 : 1 : 4), 0·62, 0·62, 0·62, in 2% AcOH, 0·58, 0·58, 0·52—0·53, in 2% AcOH (ascending), 0·50, 0·49, 0·46, and in Bu^sOH satd. with water, 0·76, —, 0·73.

(b) *Penta-acetates.* These have not yet been obtained crystalline and were unsuitable for detailed comparisons. Mollisacacidin penta-acetate, m. p. 82—87°, $[\alpha]_D^{15}$ −17·2° (1·02% in $C_2H_2Cl_4$), did not depress the m. p. of (−)-leucofisetinidin penta-acetate, m. p. 82—84°, $[\alpha]_D^{15}$ +16° (1·16% in $C_2H_2Cl_4$), and the mixture did not depress the m. p. of the (±)-penta-acetate, m. p. 82—84°, $[\alpha]_D^{15}$ −2·0° (1·49% in $C_2H_2Cl_4$), prepared from hydrogenated fustin. These amorphous materials did not melt sharply, and m. p.s here refer to the temperature of sintering and collapse. For gleditsin Dr. Mitsuno [21] reports m. p. 56—59°, $[\alpha]_D^{10}$ −7·5° (4·3% in pyridine).

(c) *Trimethyl ethers.* The m. p.s of the sesquihydrates were indefinite and appeared to depend upon the conditions (*e.g.*, rate of heating); after resolidification they had the same m. p.s as the anhydrous materials. Mollisacacidin trimethyl ether and gleditsin trimethyl ether behaved similarly, either alone or mixed together, when examined with the Leitz hot-stage microscope: m. p. 70—75° resolidification at *ca.* 100°, and m. p. 128—130° after sintering at 117—120° {cf. above; for the gleditsin ether Dr. Mitsuno [21] reports m. p. 130°, $[\alpha]_D^{10}$ +34·9° (4·5% in pyridine), and we found $[\alpha]_D^{16}$ −9·5% (0·5% in $C_2H_2Cl_4$}. A mixture of either compound with (−)-leucofisetinidin trimethyl ether similarly showed m. p. 65—70°, resolidification slowly at *ca.* 130° to a new crystalline form (racemate), m. p. 144—146°. The racemates prepared by evaporation of equimolar solutions of mollisacacidin (or gleditsin) trimethyl ether and quebracho (−)-leucofisetinidin trimethyl ether had m. p. 81—84°, resolidified at 120—130°, and then had m. p. 147—148° (in capillaries), alone or mixed with the trimethyl ether,[10] m. p. 149°, from hydrogenated fustin, and did not depress the m. p. of the (±)-7 : 3′ : 4′-trimethoxyflavan-3 : 4-diol,[11] m. p. 149°, supplied by Dr. Kulkarni (lit.,[11] m. p. 142°).

The infrared absorption of mollisacacidin, gleditsin, and quebracho (−)-leucofisetinidin trimethyl ethers in Nujol mulls and in chloroform were identical in the recorded ranges (2—15 and 2—11·5 μ). The curves from the chloroform solutions were identical with those of the two samples of racemate.[10,11] The infrared absorption curves of anhydrous mollisacacidin and gleditsin trimethyl ether were also identical (2—15 μ) when recorded with Nujol mulls of samples dried over phosphoric oxide at 110° *in vacuo*. The infrared spectra (in KBr) of mollisacacidin, (−)-leucofisetinidin, and the (±)-trimethyl ether [10] were indistinguishable.

(d) *Trimethyl ether 3 : 4-diacetates.* Mollisacacidin and quebracho (−)-leucofisetinidin trimethyl ether diacetate, $[\alpha]_D^{15}$ +16·5° (1·22% in $C_2H_2Cl_4$), had m. p. 101—102° and evaporation of an equimolar methanolic solution left a residue, m. p. 121—122° alone or mixed with the racemate supplied by Dr. A. B. Kulkarni [11] or that synthesised [10] from fustin. Infrared absorptions (in KBr) of the enantiomorphs were identical (2·5—15 μ), and closely similar to those of the two samples of racemate. The enantiomorphs (in Nujol mull) showed identical infrared absorptions (2—15 μ) which differed from that of the racemate; in carbon tetrachloride solution the enantiomorphs and the racemates [10,11] gave identical spectra (2—11·5 μ).

(e) *Trimethyl ether* iso*propylidene derivatives.* The mollisacacidin and the (−)-leucofisetinidin derivative, m. p. 120—122°, formed a racemate, m. p. 134° alone and when mixed

with the (±)-*iso*propylidene derivative described above. The infrared curves of the enantiomorphs (in KBr) were indistinguishable and closely similar to that recorded for the racemate.

We recorded also the following data. Quebracho leucofisetinidin, m. p. 130° (decomp.) (lit.,[22] 133—137°), $[\alpha]_D^{25}$ −11·1° in MeOH [lit.,[22] −12° (2%)], $[\alpha]_D^{12}$ −31·7° (0·8% in 1 : 1 acetone–water) {lit.,[22] $[\alpha]_D^{25}$ −32° (2%)}. (±)-Leucofisetinidin [ref. 10 gives m. p. 126—130° (decomp.) (dried over P_2O_5)], $[\alpha]_D^{15}$ −2·2° (0·82% in 1 : 1 acetone–water) {lit.,[10] $[\alpha]_D^{21}$ −2·4° (1·4%)}; its trimethyl ether, $[\alpha]_D^{15}$ −1·2° (0·82% in $C_2H_2Cl_4$); its trimethyl ether diacetate, m. p. 121—122° (lit., 121—122°,[10] and 122° [11]), $[\alpha]_D^{15}$ +1·2° (1·24% in $C_2H_2Cl_4$).

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