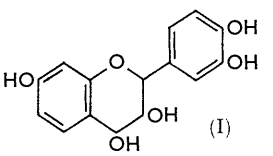
99. *Flavan Derivatives. Part IV.*[1] *Teracacidin, a New Leucoanthocyanidin from* Acacia intertexta.

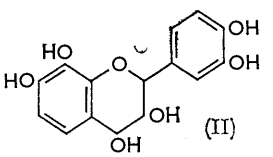
By J. W. Clark-Lewis, G. F. Katekar, and P. I. Mortimer.

Heartwood of *Acacia intertexta* contains a new leucoanthocyanidin (teracacidin) found to be (−)-7,8,4′-trihydroxy-2,3-*cis*-flavan-3,4-*cis*-diol, an analogue of melacacidin. The wood also contains a very small proportion of isoteracacidin, and (+)-pinitol. Teracacidin and isoteracacidin appear to be related to each other in the same way as melacacidin and isomelacacidin,[1] and the epimers thus differ only in configuration at the 4-position.

Acacia is a large genus of the Leguminosæ family and some two-thirds of its five [2] or eight [3] hundred species are indigenous to Australia, although apparently none is a native of New Zealand or Europe.[2] The Australian species *A. mollissima* (black wattle) is extensively cultivated in South Africa as an important source of industrial tanning material, and this species contains the leucoanthocyanidin mollisacacidin (I) isolated by Keppler [4] soon after the discovery in *A. melanoxylon* (Australian blackwood) of melacacidin (II),[5] which also occurs in *A. harpophylla* and *A. excelsa*.[1] A third member, teracacidin (III), of the *Acacia* leucoanthocyanidin series has now been isolated from *A. intertexta*,



(I)



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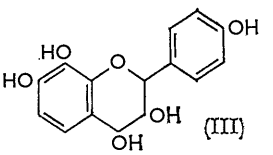


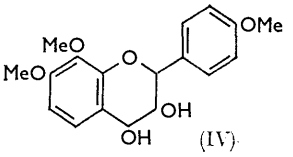
(III)

where it occurs with an isomer (isoteracacidin) which is considered to be the 4-epimer, and thus related to teracacidin in the same way as isomelacacidin [1] is related to melacacidin. It is noteworthy also that teracacidin is the first natural representative of flavonoids with the 7,8,4′-pattern of phenolic hydroxylation.

The structure of teracacidin (III) was established by oxidation of its non-phenolic trimethyl ether (IV) to 2-hydroxy-3,4-dimethoxybenzoic acid and *p*-methoxybenzoic acid, which were isolated and characterised as their methyl esters. The course of this oxidation thus corresponds to the similar reaction with melacacidin tetramethyl ether, and the leucoanthocyanidin properties of teracacidin support the flavan-3,4-diol formulation. The teracacidin structure (III) was confirmed by synthesis of the 2,3-*cis*-3,4-*cis*-racemate of 7,8,4′-trimethoxyflavan-3,4-diol (IV) by catalytic hydrogenation of 7,8,4′-trimethoxyflavonol, which gave a product with infrared absorption indistinguishable from that of the lævorotatory teracacidin trimethyl ether, but markedly different from the absorption

[1] Part III, Clark-Lewis and Mortimer, *J.*, 1960, 4106.
[2] Black, " Flora of South Australia," 2nd edn., Government Printer, Adelaide, 1948—1957.
[3] Ewart, " Flora of Victoria," Government Printer, Melbourne, 1930.
[4] Keppler, *J.*, 1957, 2721.
[5] King and Bottomley, *J.*, 1954, 1399.

of the 2,3-*trans*-3,4-*cis*-racemate prepared from *trans*-dihydro-7,8,4′-trimethoxyflavonol by reduction with sodium borohydride or by hydrogenation over palladium. Teracacidin (V; R = H) is thus stereochemically identical with melacacidin (V; R = OH).



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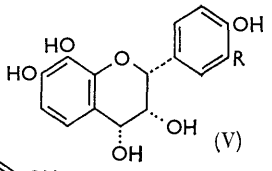

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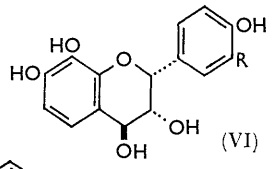

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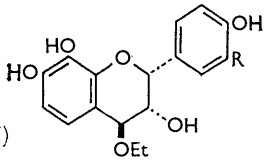
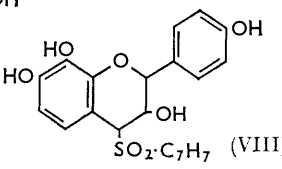


(VII)



(VIII)

The chemical and chromatographic behaviour of melacacidin (V; R = OH), isomelacacidin (VI; R = OH), and *O*-ethylisomelacacidin [1] (VII; R = OH) closely resembles the behaviour of compounds in the teracacidin series, and this indicates a similar relation between teracacidin (V; R = H), isoteracacidin (VI; R = H), and *O*-ethylisoteracacidin (VII; R = H). Thus a small proportion of isoteracacidin occurred with teracacidin, and when warmed with ethanolic acetic acid it was converted into *O*-ethylisoteracacidin (VII; R = H); this derivative was readily hydrolysed to isoteracacidin again, just as *O*-ethylisomelacacidin was readily hydrolysed to isomelacacidin. Unlike the melacacidin series, however, the teracacidin compounds have remained amorphous even when chromatographically homogeneous, and formulation of isoteracacidin as the 4-epimer (VI; R = H) and *O*-ethylisoteracacidin (VII; R = H) as its 4-ethyl ether rests largely on analogy with the melacacidin compounds. The isoteracacidin fraction, obtained by counter-current distribution of the *O*-ethyl derivative and subsequent hydrolysis, was converted by reaction with toluene-*p*-sulphinic acid into the sulphone [1] (VIII), the only crystalline phenolic compound so far obtained in the teracacidin series. This sulphone yielded also a crystalline tetra-acetate, and was converted by hot acid into the same anthocyanidin as was obtained from teracacidin, and in all these reactions isoteracacidin resembles isomelacacidin. Investigation of the teracacidin compounds was made more difficult by their occurring in the heartwood with comparatively large quantities of extractable brown polymers (" phlobaphenes ").

The stereochemical representations in the formulæ (V—VII) are supported by the similarity in molecular rotations of the methyl ethers, the sulphones, and the acetylated sulphones in the teracacidin series (—214°, —110°, —86°) with those of the melacacidin compounds (—309°, —118°, —88°), and by synthesis of the racemic trimethyl ether (IV) corresponding to (V; R = H). This synthesis was achieved by catalytic hydrogenation of 7,8,4′-trimethoxyflavonol by the method introduced for synthesising (±)-melacacidin tetramethyl ether,[6] which is the only route so far available for preparing all-*cis*-flavan-3,4-diols. Teracacidin trimethyl ether was thus obtained, but with more difficulty than for melacacidin tetramethyl ether which appears to be a very favourable case. Raney nickel catalysts with low activity leave the flavonols unaffected, and fresh W7 and W6 catalysts usually carry the hydrogenation too far through hydrogenolysis of the 4-hydroxyl group and, mainly, further hydrogenolysis to phenolic 1,3-diarylpropan-2-ols. Best yields of flavan-3,4-diols so far have attended the use of W6 catalyst stored at 0° under ethanol for 4—6 months before use.

Pinitol occurs widely in the Leguminosæ [7] and has been isolated from wood of *A.*

[6] King and Clark-Lewis, *J.*, 1955, 3384.
[7] Plouvier, *Compt. rend.*, 1955, **241**, 1838.

mollissima.[4,8] Pinitol sometimes crystallised from our acetone extracts of *A. melanoxylon* (*ca.* 0·9%) and *A. mollissima* (1·5%) heartwoods; it was also isolated from *A. intertexta* (0·3%) and *A. harpophylla* (0·05%) heartwoods, and was detected in *A. excelsa*. Demethylation of (+)-pinitol to (+)-inositol was found to occur readily in boiling 6N-hydrochloric acid, which may therefore be used for this purpose instead of hydriodic acid.[9]

Experimental

Paper chromatograms of anthocyanidins were run with Forestal solvent [10] and butanol–acetic acid–water [11] (4 : 1 : 5) was used for other phenols and for cyclitols, except where otherwise stated.

Wood specimens were collected by Mr. W. T. Jones, C.S.I.R.O., Brisbane, from botanically identified *Acacia intertexta* (herbarium number 473) and supplied by courtesy of the Chemical Research Laboratories, C.S.I.R.O., Melbourne.

Extraction of Pinitol and Leucoanthocyanidins from A. intertexta *Heartwood.*—The milled wood (3130 g.) was extracted by continuous hot percolation with light petroleum (b. p. 60—80°) for 12·hr. (4·5 g. of extractive), acetone (24 hr.), and ethanol (8 hr.); the acetone and ethanol extractives contained pinitol, teracacidin, and isoteracacidin. Evaporation of the acetone extract left a viscous residue (312 g.) which was stirred with water (2 l.) and filtered next day from a considerable quantity of amorphous deposit. The filtrate was concentrated (to 250 c.c.) under reduced pressure, and continuous extraction with ethyl acetate then yielded 22 g., 2·1 g., and 0·7 g. of polyphenolic material in successive 8 hr. periods, and left pinitol (*ca.* 17 g.) in the aqueous phase. The ethanol extract was similarly treated and gave 6·9 g. of material soluble in ethyl acetate; 4·3 g. remained in the aqueous phase. Crystallisation, from aqueous ethanol, of the pinitol fractions (from 11·5 kg. of heartwood) gave pinitol (40 g., 0·35%), m. p. 182—183° after recrystallisation from aqueous ethanol, $[\alpha]_D^{16}$ +65° (2·8% in H_2O) (Found: C, 43·0; H, 7·3; OMe, 15·6. Calc. for $C_7H_{14}O_6$: C, 43·3; H, 7·3; OMe, 16·0%). When chromatographed for 45 hr. pinitol moved 15 cm. and two unidentified polyol components moved 8·5 and 1·9 cm.

The combined ethyl acetate-soluble polyphenols (32 g.) were submitted to a 50-tube (50 c.c.) counter-current distribution between ethyl acetate and 0·067M-phosphate buffer (pH 7·0). Tubes 24—36 (peak at 29) contained teracacidin (R_F 0·46—0·54) and isoteracacidin (R_F 0·58—0·68), and the contents were collected; the ethyl acetate phases were combined with ethyl acetate extracts of the aqueous phases and dried (Na_2SO_4). Evaporation left a residue (4·8 g.) containing mainly teracacidin (R_F 0·46—0·54) and some *O*-ethylisoteracacidin (R_F 0·75—0·87) (an artefact formed during manipulation of the extracts with ethanol). A 2% aqueous solution of this residue was heated at 100° for 2 hr. and the solution then contained teracacidin (R_F 0·47—0·54) and a little isoteracacidin (R_F 0·58—0·64) but no *O*-ethylisoteracacidin (R_F 0·78—0·85); R_F values for teracacidin, isoteracacidin, and *O*-ethylisoteracacidin in 2% acetic acid were 0·41—0·52, 0·58—0·68, and 0·67—0·76. In this way, *A. intertexta* heartwood (9·1 kg.) gave a teracacidin fraction (16·8 g., tubes 24—36) and further polyphenols (80 g., tubes 37—50). The teracacidin fraction (16·8 g.) was boiled in 1% ethanolic acetic acid (170 c.c.) for 2 hr. and evaporated before counter-current distribution once more; tubes 24—40 (peak at 32) contained only teracacidin (5·1 g., 0·06%). Another sample of wood (580 g.) gave a much higher yield of teracacidin (0·5%).

Isoteracacidin Sulphone (VIII) *and its Tetra-acetate.*—Polyphenols (92 g.) from tubes 37—50 of the above counter-current distributions were heated for 2 hr. at 100° with water (600 c.c.) and acetic acid (12 c.c.), and soluble material was isolated (less than 20 g.) and converted into *O*-ethylisoteracacidin with boiling 1% ethanolic acetic acid. Evaporation left a residue which failed to crystallise and was accordingly dissolved in ethanol (23 c.c.), diluted with water (100 c.c.), and filtered from amorphous material. The filtrate was boiled for 10 min. with concentrated hydrochloric acid (0·1 c.c.), then sodium toluene-*p*-sulphinate dihydrate (10 g.) in water (20 c.c.) and acetic acid (6 c.c.) was added, and the mixture was heated on a steam-bath for 30 min. Crystallisation gave the crude sulphone (3·4 g.) and recrystallisation from

[8] Stephen, *J. Sci. Food Agric.*, 1952, **3**, 37.
[9] Stephen, *J.*, 1952, 738; cf. Maquenne, *Compt. rend.*, 1889, **109**, 813.
[10] Bate-Smith, *Biochem. J.*, 1954, **58**, 122.
[11] Partridge, *Biochem. J.*, 1948, **42**, 238.

5% acetic acid (charcoal) gave *isoteracacidin* p-*tolyl sulphone* (2·16 g.) which crystallised from aqueous acetone (44 c.c.; 1 : 1) in clusters of transparent plates which became opaque when dried in air (1·3 g.) and had m. p. 214° (decomp.), $[\alpha]_D^{24}$ −22·4° (1% in acetone) (Found: C, 60·6; H, 5·1; S, 7·3. $C_{22}H_{20}O_7S,\frac{1}{2}H_2O$ requires C, 60·4; H, 4·8; S, 7·3%). Acetylation of the sulphone (0·1 g.) with acetic anhydride (0·4 c.c.) and pyridine (2 c.c.) at room temperature during 14 hr. gave *isoteracacidin* p-*tolyl sulphone tetra-acetate*, which crystallised from methanol (2—3 c.c.) in needles (0·085 g.), m. p. 133—136° raised by recrystallisation from ethanol (67% recovery) to m. p. 137—138°, $[\alpha]_D^{24}$ −16° (1% in acetone) (Found: C, 60·3; H, 5·0; S, 5·4; Ac, 27·4. $C_{30}H_{28}O_{11}S$ requires C, 60·4; H, 4·7; S, 5·4; Ac, 28·8%).

Teracacidin (III) *and Teracacidin* 7,8,4′-*Trimethyl Ether* (IV).—Teracacidin, purified by counter-current distribution as already described, was obtained as a brown powder which gave a red colour when heated in 3N-hydrochloric acid at 100° for 15 min. The solution was extracted with pentyl alcohol, and the orange-red anthocyanidin (R_F 0·74) was compared chromatographically in Forestal solvent (6 hr.; solvent front moved 20 cm.) with cyanidin (R_F 0·55) and 3,7,8,3′,4′-pentahydroxyflavylium chloride (R_F 0·58). The anthocyanidin (R_F 0·74) similarly derived from isoteracacidin sulphone was indistinguishable from that from teracacidin.

Crude teracacidin (1·63 g.) was methylated for 5 hr. in acetone with methyl sulphate (3 g.) and potassium carbonate (10 g.); crystallisation of the product from ethanol gave *teracacidin* 7,8,4′-*trimethyl ether* in small needles (0·5 g.), m. p. 159°, $[\alpha]_D^{18}$ −65% (1% in ethanol) (Found: C, 64·9; H, 6·0; OMe, 28·0. $C_{18}H_{20}O_6$ requires C, 65·0; H, 6·1; OMe, 28·0%). The trimethyl ether was also obtained by methylating the leucoanthocyanidin with diazomethane. (−)-Teracacidin trimethyl ether gave an *isopropylidene derivative* which crystallised from methanol in needles, m. p. 139—140° (Found: C, 67·8; H, 6·5. $C_{21}H_{24}O_6$ requires C, 67·7; H, 6·5%).

Oxidation of Teracacidin Trimethyl Ether (IV).—(*a*) Powdered potassium permanganate (0·51 g.) was added gradually to teracacidin trimethyl ether (0·108 g.) in dry acetone (50 c.c.), heated on a water-bath, until the permanganate colour persisted. The deposit was collected, suspended in 5% sulphuric acid (20 c.c.), decolorised with sulphur dioxide, and heated to the b. p. before filtration. Crystallisation, and purification of the product by dissolution in aqueous sodium hydrogen carbonate (charcoal) and re-acidification, gave *p*-anisic acid (0·0166 g.), m. p. 160—177° raised to m. p. 178° (0·0121 g., 24%) by sublimation at 150° under reduced pressure. A mixture of the product with *p*-anisic acid (m. p. 180—181°) melted at 180—181°, and a mixture with veratric acid (m. p. 180°) melted at 142—145°.

(*b*) Teracacidin trimethyl ether (1·03 g.) was boiled in acetone (50 c.c.) for 4 hr. with potassium permanganate (1·5 g.). Acetone was then evaporated while water (50 c.c.) was added simultaneously. 10% Sulphuric acid (5 c.c.) was then added and manganese dioxide was dissolved with sulphur dioxide. The aqueous suspension of organic substances was extracted with ether (100 c.c. and 4 × 50 c.c.), and the combined ethereal solutions were washed with water (2 × 5 c.c.) before being extracted with 5% aqueous sodium carbonate (5 × 10 c.c.). Evaporation of the ether left a residue (0·14 g.) which gave teracacidin trimethyl ether (0·081 g.) in needles (from benzene), m. p. and mixed m. p. 159°. The sodium carbonate solution was acidified with sulphuric acid and extracted with ether (20 c.c. and 3 × 10 c.c.). Evaporation of the ether left a residue (0·67 g.) which was methylated with ethereal diazomethane (from 4 g. of nitrosomethylurea) for 5 min. The mixture was then separated with aqueous sodium hydroxide into non-phenolic and phenolic products, which gave methyl *p*-anisate (0·34 g., 76%), plates [from light petroleum (b. p. 60—80°)], m. p. and mixed m. p. 48—49°, and methyl 2-hydroxy-3,4-dimethoxybenzoate (0·29 g.) which crystallised from aqueous methanol in prisms (0·25 g., 48%), m. p. and mixed m. p. 75—76°.

(±)-7,8,4′-*Trimethoxy*-2,3-cis-*flavan*-3,4-cis-*diol* (IV).—2-Hydroxy-3,4,4′-trimethoxychalcone [12] (5 g.) was converted into 7,8,4′-trimethoxyflavonol with alkaline peroxide as described for the tetramethoxy-analogue.[5] The flavonol (3·5 g., 70%) crystallised from acetic acid in yellow needles, m. p. 195° (Kostanecki and Schreiber [12] record m. p. 198° for the compound prepared from the hydroxyiminoflavanone) (Found: C, 66·4; H, 5·0. Calc. for $C_{18}H_{16}O_6$: C, 65·9; H, 4·9%). 7,8,4′-Trimethoxyflavonol (2·0 g.) in ethanol (100 c.c.) was hydrogenated for 24 hr. at 100°/100 atm. over aged Raney nickel (W6) (*ca.* 2 g.). The suspension was filtered (kieselguhr) from the catalyst and evaporation of the filtrate under reduced pressure left a residue (1·0 g.) which was chromatographed on alumina (100 g.) deactivated with water (10 g.).

[12] Kostanecki and Schreiber, *Ber.*, 1905, **38**, 2748.

The column was developed with benzene (150 c.c.), with benzene–ether (1 : 1) (350 c.c.) which yielded an oil (0·39 g.), and then with benzene–ether (1 : 1) (150 c.c.) containing 1% of ethanol which gave the diol (0·36 g., 18%), m. p. 130—132°. (±)-7,8,4′-*Trimethoxy*-2,3-cis-*flavan*-3,4-cis-*diol* crystallised from ethanol in needles, m. p. 132—133° (Found: C, 65·1; H, 6·2. $C_{18}H_{20}O_6$ requires C, 65·1; H, 6·1%). Acetylation of the diol with acetic anhydride–pyridine at room temperature gave the *diacetate* which crystallised from ethanol in leaflets, m. p. 158—159° (Found: C, 63·3; H, 5·7. $C_{22}H_{24}O_8$ requires C, 63·5; H, 5·8%). The *isopropylidene derivative* (prepared as for the melacacidin analogue [6]) crystallised from methanol in needles, m. p. 126° (Found: C, 67·7; H, 6·5. $C_{21}H_{24}O_6$ requires C, 67·7; H, 6·5%).

(±)-2,3-trans-*Dihydro*-7,8,4′-*trimethoxyflavonol*.—Bromine (0·25 g.) in carbon tetrachloride (1 c.c.) was added to a solution of 2-acetoxy-3,4,4′-trimethoxychalcone (0·6 g.) in carbon tetrachloride (15 c.c.). After 2 hr. at room temperature the solution was evaporated under reduced pressure, and the residue was boiled with 1 : 4 aqueous acetone (10 c.c.) for 13—15 min. The solution was extracted with ether, and the ethereal solution was washed with water and then dried ($MgSO_4$). The *bromohydrin* crystallised from the filtered ethereal solution, and recrystallisation from benzene–hexane gave prisms, m. p. 138—145° (Found: C, 53·4; H, 4·8. $C_{20}H_{21}BrO_7$ requires C, 53·1; H, 4·7%). The bromohydrin was boiled with 10% aqueous sodium carbonate (8 c.c.) for 3 min. and then poured into water (50 c.c.). The precipitate was collected when solid, and crystallisation from ethanol (charcoal) gave *dihydro*-7,8,4′-*trimethoxyflavonol* (0·15 g., 28%), m. p. 172° (Found: C, 65·7; H, 5·6. $C_{18}H_{18}O_6$ requires C, 65·5; H, 5·5%). The dihydroflavonol was also prepared, without isolation of the bromohydrin, by adding aqueous sodium carbonate to the aqueous-acetone solution of the chalcone dibromide after the heating period.

(±)-7,8,4′-*Trimethoxy*-2,3-trans-*flavan*-3,4-cis-*diol*.—(*a*) Sodium borohydride (0·6 g.) was added to an ice-cold solution of the dihydroflavonol (2 g.) in methanol (*ca.* 150 c.c.), the solution was acidified with acetic acid after 24 hr., and the solvent was then removed under reduced pressure. The residual diol was dried over potassium hydroxide; it then crystallised from methanol in needles (1·3 g., 65%), m. p. 83—84°, raised to m. p. 126—127° by being dried *in vacuo* at 50° over phosphoric oxide (Found: C, 64·6; H, 6·2. $C_{18}H_{20}O_6$ requires C, 65·1; H, 6·1%). The *isopropylidene derivative* (76%) (prepared as described for the melacacidin analogue [6]) crystallised from methanol in needles, m. p. 168—169° (Found: C, 67·6; H, 6·6. $C_{21}H_{24}O_6$ requires C, 67·7; H, 6·5%).

(*b*) Dihydro-7,8,4′-trimethoxyflavonol (0·2 g.) in methanol (20 c.c.) was hydrogenated over Adams catalyst (0·01 g.) for 12 hr. at 50°/70 atm. The filtrate from the catalyst was evaporated under reduced pressure, and crystallisation of the residue from ethanol gave the diol (0·13 g., 65%) in colourless needles, m. p. 96—100° raised to m. p. 126—127° by drying at 50° over phosphoric oxide *in vacuo*. It did not depress the m. p. of the diol prepared by method (*a*).

(+)-*Inositol from* (+)-*Pinitol*.—(+)-Pinitol from *A. intertexta* had m. p. 184—185°, $[\alpha]_D^{25}$ +65° (3% in H_2O) (lit.,[13] m. p. 186—188°, $[\alpha]_D^{25}$ +65·5° in H_2O). Paper chromatography of the reaction mixture showed that conversion of pinitol (R_F 0·23) into inositol (R_F 0·14) occurred rapidly in boiling 6N-hydrochloric acid, and was complete in 8 hr.; no other product was detected with the alkaline silver nitrate spray. (+)-Pinitol (5 g.) was boiled with 6N-hydrochloric acid for 8 hr. and the pale brown solution was evaporated to dryness on a steam-bath. The residue was re-evaporated with water twice to remove acid, and then with ethanol, which left a crystalline residue (4·75 g.), m. p. 194—221°. Recrystallisation from aqueous ethanol gave (+)-inositol as prisms (4·1 g., 88%), m. p. 236—238° raised by recrystallisation to m. p. 239—240°, $[\alpha]_D^{16}$ +64° (1·2% in H_2O) (Found: C, 39·8; H, 6·7. Calc. for $C_6H_{12}O_6$: C, 40·0; H, 6·7%). Stephen [9] records m. p. 244° and $[\alpha]_D^{28}$ +66·2° (1·21% in H_2O) for (+)-inositol obtained by demethylation of (+)-pinitol with hydriodic acid.

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UNIVERSITY OF ADELAIDE, SOUTH AUSTRALIA. [*Received, May 30th*, 1960.]

[13] Karrer, "Konstitution und Vorkommen der organischen Pflanzenstoffe," Birkhäuser Verlag, Basle, 1958, p. 117.