525. *The Isolation and Constitution of Mollisacacidin, a New* leuco*Anthocyanidin from the Heartwood of* Acacia mollisima *Willd.*

By H. H. KEPPLER.

A new *leuco*anthocyanidin, mollisacacidin, has been isolated from the heartwood of *Acacia mollisima* (black wattle). Oxidation by periodic acid of its trimethyl ether revealed the presence of a glycol group, which is shown to be *cis*. With potassium permanganate in acetone this ether yielded 4-methoxysalicylic and veratric acid. The structure of the *leuco*anthocyanidin is thus postulated as *cis*-3 : 4 : 7 : 3′ : 4′-pentahydroxyflavan, which is confirmed by reduction of fustin (isolated from the heartwood of *Rhus glabra*) to mollisacacidin. The natural and synthetic *leuco*anthocyanidin both give fisetinidin chloride on treatment with mineral acid.

THE first reported isolation of a crystalline *leuco*anthocyanin from a natural source was the recent instance of one from *Acacia mollisima* (black wattle).[1] King and Bottomley[2] obtained an amorphous *leuco*anthocyanidin from *Acacia melanoxylon*, but fully characterised the compound with crystalline derivatives. In the present investigation, black wattle heartwood was extracted with hot acetone. A light brown fraction therefrom, soluble in hot water and in cold acetone, was polyphenolic since it gave a green colour with ferric chloride. Two-dimensional paper chromatography disclosed two main components. Column chromatography on Solka-floc (B.W. 200 grade) with water as developer yielded two compounds giving characteristic *leuco*anthocyanidin tests;[3] one (in small yield) was amorphous; the other crystallised and is named mollisacacidin. The amorphous *leuco*-anthocyanidine had the same R_F as (+)-catechin, but had absorption maxima at 280 and 310 mμ whereas catechin had only one (280 mμ): the crystalline *leuco*anthocyanidin had maxima at 230 and 280 mμ. Both *leuco*anthocyanidins are non-glycosidic, and both are converted into the same anthocyanidin, R_F 0·69, by 10% ethanolic hydrochloric acid. It is considered that the amorphous compound may be a polymer of the crystalline monomer. With methyl sulphate and potassium carbonate in acetone or with diazomethane, mollisacacidin gave a crystalline trimethyl ether, and with acetic anhydride a penta-acetate. It thus contains five hydroxyl groups, three of them phenolic. Mitchell's colorimetric method[4] showed the presence of two *ortho*-phenolic hydroxyl groups.

Periodic acid at room temperature rapidly oxidised mollisacacidin trimethyl ether, two equivalents of oxygen being consumed. An αβ-glycol structure is thus shown, and the *cis*-configuration was indicated by formation of an acidic borate complex by the methylated *leuco*anthocyanidin with sodium borate.[5] With potassium permanganate, mollisacacidin trimethyl ether gave 4-methoxysalicylic and veratric acid, identified by m. p.s and infra-red spectra over the skeletal range 700—1700 cm.$^{-1}$.

The structure as *cis*-3 : 4 : 7 : 3′ : 4′-pentahydroxyflavan was then conclusively proved by catalytic conversion of fustin (3 : 7 : 3′ : 4′-tetrahydroxyflavanone), isolated from the heartwood of *Rhus glabra*, into mollisacacidin. Catalytic reduction of this type of compound should favour formation of the *cis*-glycol.[6] Further, both the natural and the synthetic *leuco*anthocyanidin gave fisetinidin chloride[7] on treatment with 3N-hydrochloric acid in hot propan-2-ol.[8] Whereas several solvent systems failed to differentiate between fisetinidin and pelargonidin chloride on paper chromatography, Geissman and Jurd's

[1] Keppler, *Chem. and Ind.*, 1956, 380.
[2] King and Bottomley, *ibid.*, 1953, 1368; *J.*, 1954, 1399.
[3] Bate-Smith, *Biochem. J.*, 1954, **58**, 122.
[4] Mitchell, *Analyst*, 1923, **48**, 2; 1924, **49**, 162; *J. Internat. Soc. Leather Trades' Chemists*, 1936, **20**, 99.
[5] Boëseken and Hermans, *Rec. Trav. chim.*, 1921, **40**, 525.
[6] Joshi and Kulkarni, *Chem. and Ind.*, 1954, 1456; *J.*, 1953, 1027; Hückel, *Annalen*, 1937, **533**, 1.
[7] Freudenberg and Maitland, *Annalen*, 1934, **510**, 193.
[8] Hillis, *J. Internat. Soc. Leather Trades' Chemists*, 1954, **38**, 91.

spectrographic method[9] achieved this; it is based on the ability of anthocyanidins having adjacent phenolic hydroxyl groups to form a complex with aluminium in neutral solution.

A second fraction of the crude extract contained pinitol, an inositol methyl ether previously found in wattle wood by Stephen [10] and a common constituent of conifers.[11] A third fraction yielded a crystalline compound agreeing in analysis with the steroid isolated by Stephen [10] from wattle wood but differing from it in its Liebermann–Burchard colour reaction and in the analysis of its monoacetate.

Experimental

Extraction of Heartwood.—Air-dried ground wattle heartwood (2 kg.) was extracted with acetone (4 l.) under reflux for 4 hr. After three extractions, the combined filtrates were evaporated at 50° under a reduced pressure of nitrogen. The dark brown residue was fractionated by repeated extraction with hot water. Insoluble material (A) was set aside. The solution of the water-soluble material (B) was evaporated at 80° under a reduced pressure of nitrogen, giving a dark brown powder (25 g.). This was triturated with cold anhydrous acetone; the soluble material was isolated as above and comprised fraction I. Acetone-insoluble material comprised fraction II. The water-insoluble material (A) was extracted (Soxhlet) with ether (500 ml.) for 6 hr. The yellow ethereal solution was evaporated under reduced pressure to give a light yellow residue (fraction III).

Fraction I.—(a) *Paper chromatography.* A portion (0·1 g.) in acetone (5 ml.) was chromatographed on Whatman's No. 2 paper, with aqueous 6% acetic acid as the one-way developer. The developed chromatogram was dried and dipped in 1 : 1 aqueous 0·3% ferric chloride and 0·3% potassium ferricyanide. Apart from a certain degree of trailing from the origin of the chromatogram, two compounds, R_F 0·65 and 0·53, staining blue, were revealed.

(b) *Separation of the components.* " Solka-floc " in 6% aqueous acetic acid was placed in a Pyrex-glass column (2″ × 36″), and after settling was washed with 6% aqueous acetic acid, and then with distilled water until free from acid. Fraction I (16 g.) in cold water (40 ml.) was added and the column developed with water; " break through " of phenolic material was found by the ferric chloride–ferricyanide reagent. The fractions were collected and analysed on paper as above. Fractions 1—3 contained no polyphenols. Fractions 4—5 contained a phenol of R_F 0·64, and fractions 6—8 contained this phenol and another, of R_F 0·53. Fractions 9 and 10 contained only traces thereof.

Fractions 4—8 were combined and evaporated at 50° under a reduced pressure of nitrogen to 20 ml., then chromatographed as previously, but on a smaller column. The two compounds, R_F 0·64 and 0·53, were thus completely separated.

(c) *Compound,* R_F 0·53. The appropriate solutions were evaporated to 15 ml. No crystallisation occurred, even after 72 hr. Evaporation to dryness at 50° as above gave a light brown powder.

(d) *Mollisacacidin,* R_F 0·64. The combined solutions containing this compound were evaporated to 20 ml. and crystallised after 24 hr. The colourless *compound* was recrystallised several times from hot distilled water, colourless needles being obtained. The purified material gradually reddens above 110°, melts indefinitely at 125—130°, and has $[\alpha]_D^{18}$ 12·6° (*c* 1 in MeOH), $\lambda_{max.}$ 230, 280 mμ (log ε 4·26, 3·83), $\lambda_{min.}$ 254 mμ (log ε 2·92) (Found, in a sample dried at 56°/0·2 mm.: C, 55·5; H, 5·7. $C_{15}H_{14}O_6,2H_2O$ requires C, 55·2; H, 5·5%).

Methylation of Mollisacacidin.—(*a*) Mollisacacidin (1·5 g.) and diazomethane (from nitrosomethylurea, 20 g.) in methanol (100 ml.) were kept at 0° for 24 hr., then treated with acetic acid, and evaporated under reduced pressure. The residue, in ether, was washed with 4% aqueous sodium hydroxide and then water, and evaporated to an almost colourless residue (1·2 g.). Recrystallisation of this from ether–light petroleum (b. p. 40—60°) afforded the *ether* as needles. m. p. 129° (sinter 76—77°) [Found, in a sample dried at 65°/0·2 mm.: C, 64·4; H, 6·3; OMe, 27·0%; *M* (Rast), 337. $C_{18}H_{20}O_6$ requires C, 65·0; H, 6·1; 3OMe, 28·0%; *M*, 332].

(*b*) To mollisacacidin (0·5 g.) in anhydrous acetone (70 ml.), containing anhydrous potassium

[9] Geissman and Jurd, *Arch. Biochem. Biophys.*, 1955, **56**, 259.
[10] Stephen, *J. Sci. Food Agric.*, 1952, **3**, 37.
[11] Plouvier, *Compt. rend.*, 1953, **236**, 317.

hydrogen carbonate (7 g.), dimethyl sulphate (1·5 ml.) was gradually added with stirring. The whole was heated on a water-bath for 4 hr., filtered, and evaporated under reduced pressure. The yellow residue was treated in benzene with charcoal and stirred into an excess of light petroleum (b. p. 40—60°). The white flocculent precipitate recrystallised from ether as needles (130 mg.), m. p. 128—130° alone or mixed with the material from (*a*).

Acetylation of Mollisacacidin.—Mollisacacidin (200 mg.), pyridine (3 ml.), and acetic anhydride (2 ml.) were kept overnight at room temperature, then heated on a water-bath for 15 min. and poured into an excess of cold water. The precipitated *acetate* was repeatedly dissolved in ether and reprecipitated with light petroleum (Found, in a sample dried at 100°/0·2 mm. : C, 60·0; H, 5·1; Ac, 42·2. $C_{25}H_{24}O_{11}$ requires C, 60·0; H, 4·8; Ac, 43·0%).

The Glycol Group.—Mollisacacidin trimethyl ether (34·6 mg.) and catechin tetramethyl ether (35·5 mg.) were dissolved separately in methanol (50 ml.). To each, in turn, were added 2 ml. of 0·5M-periodic acid and each whole diluted to 100 ml. A control (100 ml.), of 2 ml. of 0·5M-periodic acid in methanol, was also prepared. Aliquot parts (10 ml.) were withdrawn at intervals. Before titration, cold saturated sodium hydrogen carbonate solution (5 ml.) was added. The iodine liberated on addition of potassium iodide was titrated against 0·01N-arsenious oxide. After 2·5 hr. 1·02 mols. of periodate were consumed, but this gradually increased, being 1·09 mols. after 20 hr. There was no absorption with catechin or the blank control solution. After 3 hr., methylated catechin started to crystallise, and work with this ceased.

A solution (50 ml.) of mollisacacidin trimethyl ether (20 mg.) in 50% aqueous ethanol had pH 5·8. The pH was adjusted to 10·6 with sodium carbonate solution. A solution (pH 10·8) of sodium borate (27 mg.) in 50% aqueous ethanol (100 ml.) was then added in portions. On addition of 0·6—1 ml. the pH fell to 8·5.

Oxidation with Potassium Permanganate.—Mollisacacidin trimethyl ether (1 g.), potassium permanganate (2·5 g.), and acetone (100 ml.) were heated under reflux for 4 hr., water was added, and the acetone evaporated under reduced pressure. The aqueous suspension was saturated with sulphur dioxide and extracted with ether. The acids were taken up with aqueous sodium hydrogen carbonate, recovered by acidification, and treated in dry ether for 2 hr. with excess of diazomethane in 1 : 1 ether–methanol. The methylated derivatives were recovered and triturated with 2N-sodium hydroxide solution.

An alkali-soluble ester A (m. p. 150—153°) was precipitated with hydrochloric acid and treated with potassium hydroxide (0·7 g.) in ethanol (10 ml.) and water (10 ml.) on a water-bath for 2 hr. The alcohol was removed and the solution diluted with water and extracted with ether. Evaporation of the ether afforded a white residue (150 mg.), which was dried ($CaCl_2$) and sublimed, giving fractions (i) (95 mg.), sublimes at 90—95°/0·2 mm., m. p. 144—148°, and (ii) (10 mg.), sublimes at 105—115°/0·2 mm., m. p. 164—168°. With 5% aqueous ferric chloride fraction (ii) gave no colour, whereas fraction (i) gave a wine-red colour. Further sublimation of fraction (i) at 70—72°/0·2 mm. afforded a colourless sublimate, m. p. 154—156° alone or mixed with synthetic 4-methoxysalicylic acid.

The alkali-insoluble ester B, after being kept for 24 hr. over silica gel, crystallised (250 mg.; m. p. 48—53°). It was hydrolysed with potassium hydroxide (1·2 g.) in water (20 ml.) and ethanol (15 ml.). After 3 hr., the free acid was recovered as previously and sublimed, giving three fractions. The last of these, when resublimed, had m. p. 182—184° unchanged and did not depress the m. p. of synthetic veratric acid.

Fustin from the Heartwood of Rhus glabra.—The finely ground air-dried heartwood (900 g.) was extracted twice with ethyl acetate (2 × 4 l.) under reflux for 4 hr. The combined solutions were filtered and evaporated at 60° under reduced pressure to a yellow residue (36 g.). Paper chromatography as above showed the presence of components of R_F 0·45 and 0·67, both staining blue.

The extract (5 g.) was treated with hot water (50 ml.) and filtered [insoluble material (X)]. The filtrate was chromatographed on a column of Solka-floc; elution with 6% aqueous acetic acid gave ten fractions (20 ml. each). Paper chromatography showed a clear separation of the component of R_F 0·45. The solutions (120 ml.) containing this were evaporated at 60° to 30 ml. and crystallised in 24 hr. Several recrystallisations from hot water gave fustin as colourless needles (0·9 g.), m. p. and mixed m. p. 213—215°, $\lambda_{max.}$ 235, 278, 310 mμ ($E^{1\%}_{1cm.}$ 657, 583, 268), $\lambda_{min.}$ 225, 250, 305 mμ ($E^{1\%}_{1cm.}$ 624, 180, 265) (Found, in a sample dried at 100°/0·2 mm. : C, 62·3; H, 4·1. Calc. for fustin $C_{15}H_{12}O_6$: C, 62·5; H, 4·2%). Methylated with diazomethane,

as previously, fustin readily formed a trimethyl ether, needles, m. p. 141—142° (from methanol) (lit., 143—144°).

The yellow insoluble material (X) was dissolved in a minimum volume of boiling methanol and rapidly filtered. Cooling gave yellow needles of fisetin, m. p. 350°, $\lambda_{max.}$ 316, 365 mμ ($E^{1\%}_{1cm.}$ 421, 770), $\lambda_{min.}$ 280, 325 mμ ($E^{1\%}_{1cm.}$ 164, 383).

Catalytic Reduction of Fustin.—Fustin (1·4 g.) in absolute methanol (100 ml.) containing platinum oxide (0·1 g.) absorbed 130 ml. of hydrogen in 4 hr. The solution was filtered, evaporated, and analysed on Whatman's No. 2 filter paper with aqueous 6% acetic acid as developer. Fustin and mollisacacidin were chromatographed alongside the reduction product(s). The developed chromatogram showed unchanged fustin (R_F 0·45) and a product with the same R_F as mollisacacidin (0·64). Accordingly, the solvent was removed and the residue dissolved in ethyl acetate (100 ml.) and extracted with water. Evaporation of the aqueous phase yielded a colourless residue (0·6 g.), recrystallisation of which from hot water gave needles with an indefinite m. p. Above 130°, the compound rapidly reddens (Found, in a sample dried at 56°/0·2 mm.: C, 55·6; H, 5·3. Calc. for $C_{15}H_{14}O_6,2H_2O$: C, 55·2; H, 5·5%). Methylated with diazomethane, the compound formed a trimethyl ether, m. p. 129° (sinter 76—77°), alone or mixed with mollisacacidin trimethyl ether. The infrared spectra of the synthetic compound and mollisacacidin over the skeletal range 700—1700 cm.$^{-1}$ were identical.

Conversion of the leuco*Anthocyanidins into Fisetinidin Chloride.*—Mollisacacidin (0·3 g.) was refluxed with 2 ml. of a 5 : 1 mixture of propan-2-ol and 3N-hydrochloric acid for 1 hr., then evaporated at 80° under reduced pressure. A portion (83 mg.) of the red amorphous residue was applied in alcohol (1 ml.) to Whatman's No. 3 MM. filter paper. The paper was developed with acetic acid–water–concentrated hydrochloric acid (30 : 10 : 3). After 18 hr., the band of anthocyanidin was cut out and the anthocyanidin recovered by elution with methanol. Evaporation of the solvent gave the chromatographically pure product (6·8 mg.). The anthocyanidin was compared against the anthocyanidin produced from the synthetic *leuco*anthocyanidin, and against fisetinidin chloride and pelargonidin. All four showed a pink spot of R_F 0·67—0·69 with the above solvent mixture. Pelargonidin had $\lambda_{max.}$ 530 mμ unaffected by addition of 0·2% aluminium chloride solution, but the other three compounds changed colour from red to blue and showed $\lambda_{max.}$ shifts from 525 to 570 mμ on formation of the aluminium complex.

Isolation of Pinitol.—Fraction II, in aqueous ethanol, gave white rhombs, m. p. 185—186·5°, alone or mixed with pinitol.

Isolation of the "Steroid."—Fraction III was shaken in ether with 4% sodium hydroxide solution, then with water, dried (Na_2SO_4), and evaporated at room temperature. The gummy residue was triturated with light petroleum (b. p. 40—60°). A white amorphous insoluble residue (1·2 g.) crystallised from 95% methanol as needles, m. p. 158—160° (1·0 g.). These were dried and treated with acetic anhydride (10 ml.) in pyridine (15 ml.) on a boiling-water bath. After 30 min. excess of anhydride was destroyed and the *acetate* recovered. Recrystallised several times from methanol it formed colourless plates, m. p. 167—169° (Found, in a sample dried at 100°/0·2 mm.: C, 81·0; H, 11·3. $C_{28}H_{48}O_2$ requires C, 80·8; H, 11·5. $C_{29}H_{50}O_2$ requires C, 80·9; H, 11·7%). The acetate (0·3 g.) was hydrolysed for 30 min. with boiling 10% alcoholic potassium hydroxide, poured into water, and extracted with ether. Evaporation of the ether yielded the colourless *product*, which recrystallised from methanol in colourless needles, m. p. 160—161°, $\lambda_{max.}$ 260 mμ (log ε 2·97), $\lambda_{min.}$ 238 mμ (log ε 2·16) (Found, in a sample dried at 100°/0·2 mm.: C, 83·0; H, 12·0. $C_{26}H_{46}O$ requires C, 83·35; H, 12·4. $C_{27}H_{48}O$ requires C, 83·4; H, 12·45%).

The author thanks Dr. T. White, Forestal Central Laboratories, England, and Professor F. L. Warren, University of Natal, South Africa, for their interest and advice, also the Management of the Natal Tanning Extract Company, Limited, for permission to publish this paper.

CENTRAL LABORATORIES, NATAL TANNING EXTRACT CO. LTD.,
PIETERMARITZBURG, NATAL, S. AFRICA. [*Received, December 11th,* 1956.]