76. *Toxic Constituents of the Australian Finger Cherry,* Rhodomyrtus macrocarpa *Benth.*

By S. Trippett.

Rhodomyrtoxin, the toxic constituent of the immature Australian finger cherry, has been isolated and shown to be a tetrahydroxydimethyldi*iso*valeryldibenzofuran.

The Australian finger cherry, *Rhodomyrtus macrocarpa* Benth., is widely distributed in the more tropical areas of Australia and in New Guinea. The bright red fruit (approx. ½ in. × 1 in.), although unpalatable, has long been suspected of causing permanent blindness when eaten, and of poisoning stock.[1] Medical evidence is conflicting and the suggestion has been made that a fungus associated with the fruit is responsible for these effects. A supply of the dried immature fruit was made available by the Commonwealth of Australia Scientific and Industrial Research Organisation, and this has been examined for toxic constituents.

Solvent extraction of the fruit readily gave, in up to 1% yield, the yellow toxin, rhodomyrtoxin, LD_{50} 12 mg. per kg. (mice). In alkali, rhodomyrtoxin gave an orange solution which slowly absorbed oxygen and became green, while in concentrated sulphuric acid the

[1] L. J. Webb, "Guide to the Medicinal and Poisonous Plants of Queensland," Bull. 232, Council for Scientific and Industrial Research, Melbourne, 1948.

solution was red changing to green on warming. With alcoholic ferric chloride a brown-green precipitate was formed. Rhodomyrtoxin was not reduced in the presence of a palladium catalyst, contained no methoxyl group, and gave no carbonyl derivatives.

Analysis of rhodomyrtoxin and of its colourless acyl derivatives suggested the formula $C_{24}H_{28}O_7$ containing four acylatable hydroxyl groups, although this formula was firmly established only after much of the work described below. The molecular weight determined by the Rast method was 380; the theoretical value is 426, but an error of 12% is not unusual in these determinations. Rhodomyrtoxin showed a strong band in the infrared spectrum at 6·2 μ, while its acyl derivatives absorbed strongly at 5·85 μ: this behaviour is characteristic of acylphloroglucinol groups and the presence of two such groups in rhodomyrtoxin was confirmed by the formation of 1·8 mols. of *iso*valeric acid when the toxin was heated with 90% sulphuric acid, and of 1·35 mols. of *iso*valeraldehyde on reduction with sodium amalgam.[2] Alkali-fusion of rhodomyrtoxin gave phloroglucinol; the molecule therefore contains two *iso*valeryl groups attached to a potential phloroglucinol nucleus.

Clemmensen reduction of rhodomyrtoxin, or high-pressure reduction at 100° in the presence of Raney nickel, gave a product which rapidly absorbed oxygen and was isolated as the stable tetra-acetate, $C_{24}H_{28}O(OAc)_4$. When refluxed with constant-boiling hydriodic acid the toxin gave dide*iso*valerylrhodomyrtoxin, $C_{14}H_{12}O_5$, which in alkali rapidly absorbed oxygen giving a bright green solution fading to orange. It gave a stable tetra-acetate, $C_{22}H_{20}O_9$, whose ultraviolet spectrum was similar to that of the acetate obtained after Clemmensen reduction. With diazomethane a trimethyl ether, $C_{17}H_{18}O_5$, was formed which was insoluble in alkali and gave no colour with ferric chloride. Further methylation with potassium carbonate and methyl iodide gave a tetramethyl ether, $C_{18}H_{20}O_5$. The trimethyl ether was stable to chromic acid at room temperature and its monoacetate showed absorption in the infrared at 5·67 μ, characteristic of an enol-acetate. The fourth hydroxyl group is therefore regarded as phenolic in spite of its general lack of phenolic character. The trimethyl ether was unaffected by lithium aluminium hydride and showed no infrared absorption in the carbonyl region. The seventh oxygen of rhodomyrtoxin must therefore be an ether-oxygen stable to refluxing hydriodic acid, *i.e.*, probably in a diphenyl ether group.

Oxidation of rhodomyrtoxin with alkaline hydrogen peroxide gave methylmalonic acid, while *C*-methyl determinations on the trimethyl ether indicated the presence of at least two *C*-methyl groups. On this basis rhodomyrtoxin becomes $C_{12}O(OH)_4Me_2(COR)_2$, and is most readily formulated as a tetrahydroxydimethyldi*iso*valeryldibenzofuran. This formulation is supported by a comparison of the ultraviolet spectrum of the tetra-acetate $C_{22}H_{20}O_9$ with that of 1 : 3 : 7 : 9-tetra-acetoxydibenzofuran.

Many attempts were made to obtain chemical evidence for the presence of a dibenzofuran nucleus. Oxidation of the trimethyl ether $C_{17}H_{18}O_5$ and the tetramethyl ether led to complete breakdown, while treatment of the tetramethyl ether with sodium in liquid ammonia, which splits diphenyl ethers,[3] gave partial demethylation with no fission of the diphenyl ether bridge. Finally, distillation of the tetra-acetate $C_{22}H_{20}O_9$ with zinc dust gave, in 0·5% yield, an oil which behaved on chromatography as a hydrocarbon. The ultraviolet ($\lambda_{max.}$ 252, 282 mμ; ε 15,200, 11,800) and infrared ($\lambda_{max.}$ 8·38, 13·3 μ) spectra showed this to be essentially a (di)methyldibenzofuran, and comparison of the spectra with those of the four methyldibenzofurans and of the six dimethyldibenzofurans having both methyls in the same ring [4] showed it to be 4-methyldibenzofuran ($\lambda_{max.}$ 252, 282 mμ; ε 15,700, 12,400. $\lambda_{max.}$ 8·38, 13·3 μ). Loss of a methyl group has previously been noted in zinc dust distillations.[5]

Because of the strongly acidic conditions used in removing the *iso*valeryl groups from

[2] Birch and Todd, *J.*, 1952, 3102.
[3] Birch, *Quart. Rev.*, 1950, **4**, 69.
[4] Unpublished work.
[5] Hochstein, Stephens, Conover, Regna, Pasternack, Gordon, Pilgrim, Brunings, and Woodward, *J. Amer. Chem. Soc.*, 1953, **75**, 5455.

rhodomyrtoxin, the possibility of rearrangement of the carbon skeleton was investigated. Reduction of rhodomyrtoxin tetramethyl ether by lithium aluminium hydride gave a diol, $C_{28}H_{40}O_7$, whose ultraviolet spectrum was similar to that of the tetramethyl ether $C_{18}H_{20}O_5$. Rhodomyrtoxin itself is therefore based on a dibenzofuran nucleus. Further, if the suggested formulation of the toxin as a fully substituted dibenzofuran were correct, dide*iso*valerylrhodomyrtoxin should readily be reconverted into rhodomyrtoxin. Treatment of dide*iso*valerylrhodomyrtoxin with boron trifluoride in acetic acid gave an orange diacetyl compound which resembled rhodomyrtoxin in its chemical behaviour and in its ultraviolet and infrared spectra. However, a similar reaction with boron trifluoride in *iso*valeric acid gave a di*iso*valeryl compound which was very similar to, but not identical with, rhodomyrtoxin. This *iso*rhodomyrtoxin (for ultraviolet spectrum see Fig. 2) gave a colourless tetra-acetate and had an infrared spectrum virtually identical with that of

FIG. 1. *Light absorption of tetra-acetoxy-dide*isovalerylrhodomyrtoxin *(A) and 1 : 3 : 7 : 9-tetrahydroxydibenzofuran (B) in ethanol.*

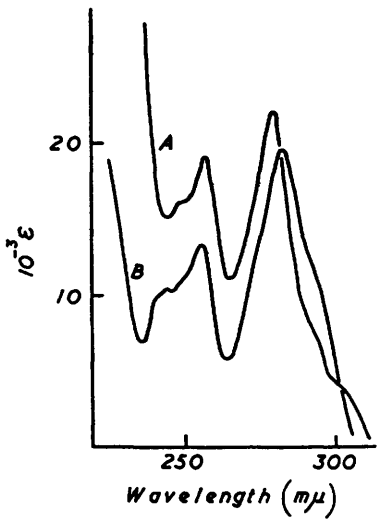
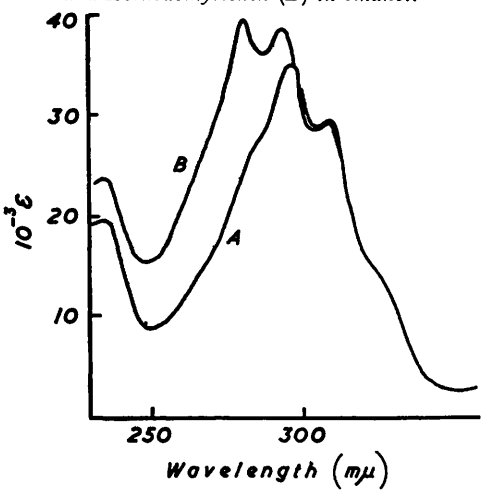


FIG. 2. *Light absorption of rhodomyrtoxin (A) and* iso*rhodomyrtoxin (B) in ethanol.*

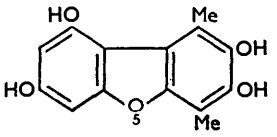


rhodomyrtoxin. Rhodomyrtoxin was itself unaffected by boron trifluoride in *iso*valeric acid; a rearrangement must therefore have occurred during removal of the *iso*valeryl groups from the toxin with hydriodic acid. This explains why it was not possible to isolate the tetra-acetate $C_{22}H_{20}O_9$ after reduction of rhodomyrtoxin with sodium amalgam.

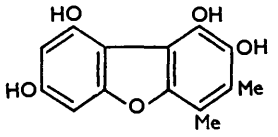
There are three possible formulæ (I—III) for dide*iso*valerylrhodomyrtoxin. The most symmetrical formula (III) would explain why hydrogen-bonding by *iso*valeryl groups of the hydroxyls in one ring should have such a marked stabilising effect on the lability of the molecule towards oxygen, the instability in this case being due to a 4 : 4′-dihydroxydiphenyl system. Formula (III) is also supported by the ultraviolet spectra of Fig. 1 and by the behaviour of 1 : 3 : 7 : 9-tetrahydroxydibenzofuran in alkali which closely parallels that of dide*iso*valerylrhodomyrtoxin. It does not, however, readily account for the formation of *iso*rhodomyrtoxin, which is most readily explained on the basis of an opening of the furan ring followed by re-closure on a different position during treatment with hydriodic acid. This is possible only with formula (II) which would arise in this way from 1 : 4 : 7 : 9-tetrahydroxy-2 : 3-dimethyldibenzofuran. Synthetic experiments are in hand to decide between the formulæ (I—III).

Extraction of a small quantity of fresh, mature finger cherries gave a substance isomeric

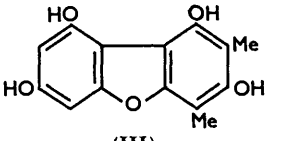
with rhodomyrtoxin and showing similar chemical behaviour (see Experimental section) but differing in its ultraviolet spectrum. The small amount of material available precluded further work on this compound.



(I)



(II)



(III)

Experimental

Except where otherwise stated, ultraviolet spectra are taken in 95% ethanol. Light petroleum refers to the fraction b. p. 40—60°.

Isolation of Rhodomyrtoxin.—Dried immature fruit (1200 g.), ground to a coarse powder, was extracted at room temperature with acetone until further extracts were almost colourless. The combined extracts were evaporated below 40°, the residue dissolved in ether (500 ml.), and light petroleum (1500 ml.) added. The resulting suspension was filtered, and the filtrate evaporated on the water-bath until solid began to separate, then set aside at room temperature for several days. Filtration gave crude *rhodomyrtoxin* (15—23 g.). Recrystallisation from acetic acid (twice) and from benzene (thrice) gave pure material, m. p. 198—199°, $\lambda_{max.}$ 234, 296, 307, 395 mμ (ε 19,700, 35,600, 29,000, 4060) (Found : C, 67·3, 67·2; H, 6·4, 6·4. $C_{24}H_{28}O_7$ requires C, 67·3; H, 6·6%).

Treatment with pyridine–acetic anhydride overnight at room temperature gave the *tetra-acetate*, m. p. (from butan-1-ol) 191—192°, $\lambda_{max.}$ 250 mμ (ε 53,000) (Found : C, 64·8; H, 6·3. $C_{32}H_{36}O_{11}$ requires C, 64·5; H, 6·05%). The following derivatives were also prepared : *tetrabenzoate*, m. p. (from butan-1-ol) 230—233° (Found : C, 74·0; H, 5·15. $C_{52}H_{44}O_{11}$ requires C, 74·0; H, 5·2%); *tetratoluene-p-sulphonate*, m. p. (from butan-1-ol) 170—171° (Found : C, 59·7; H, 5·1; S, 12·0. $C_{52}H_{52}O_{15}S_4$ requires C, 59·7; H, 5·0; S, 12·2%); and *tetra-p-chlorobenzoate*, m. p. (from ethanol) 228° (Found : C, 63·7; H, 3·5; Cl, 14·7. $C_{52}H_{40}O_{11}Cl_4$ requires C, 63·6; H, 4·1; Cl, 14·5%).

Repeated methylation of rhodomyrtoxin with dimethyl sulphate and alkali gave the colourless syrupy *tetramethyl ether* (purified by chromatography), $\lambda_{max.}$ 252, 294 mμ (ε 37,000, 17,000), $\lambda_{max.}$ 5·87 μ [Found : OMe, 24·5. $C_{24}H_{24}O_3(OMe)_4$ requires OMe, 25·2%].

When heated with 90% sulphuric acid at 100° for 20 min., rhodomyrtoxin gave 1·8 mol. of volatile acid. Isolation of this gave *iso*valeric acid, identified by infrared spectrum and by formation of the piperazine salt, m. p. 137—138° (Found : N, 9·6. Calc. for $C_{14}H_{30}O_4N_2$: N, 9·7%) undepressed on mixture with piperazinium di*iso*valerate, m. p. 138—139°.

Reduction of Rhodomyrtoxin with Sodium Amalgam.—Rhodomyrtoxin (0·5 g.) in 10% aqueous sodium hydroxide (10 ml.) was treated with 3% sodium amalgam (15 g.) for 3 hr. The solution was then decanted, diluted with water (100 ml.), and distilled rapidly for 10 min. The distillate on treatment with a saturated solution of 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid gave *iso*valeraldehyde 2 : 4-dinitrophenylhydrazone (368 mg.), m. p. and mixed m. p. 124°.

Alkali Fusion of Rhodomyrtoxin.—Rhodomyrtoxin (0·25 g.) was fused in a nickel crucible with sodium hydroxide (4 g.) and a few drops of water for ½ hr. The cooled melt was dissolved in water, and the solution acidified with sulphuric acid and continuously extracted with ether. The extract, on paper chromatography in butan-1-ol–acetic acid–water (4 : 1 : 5) and in *m*-cresol, showed the presence of phloroglucinol, identified by the characteristic behaviour with ammonia vapour, ferric chloride, and with vanillin. Methylphloroglucinol was absent. Sublimation of the extract at 10^{-4} mm. gave phloroglucinol, m. p. and mixed m. p. 213—217° (rapid heating).

Oxidation of Rhodomyrtoxin with Alkaline Hydrogen Peroxide.—Hydrogen peroxide (50 ml.; 20-vol.) was added to rhodomyrtoxin (0·5 g.) in 5% aqueous potassium hydroxide (50 ml.), the solution set aside at room temperature for 23 hr., then at 100° for 1 hr., neutralised with hydrochloric acid, and then made just alkaline with ammonia, and oxalic acid removed by the addition of saturated calcium chloride solution (2 ml.). After filtration and acidification, the solution was continuously extracted with ether and the extract sublimed at 120°/10^{-4} mm., to

give methylmalonic acid (43 mg.), m. p. 125—130° with the evolution of gas. This m. p. behaviour, while variable and unreliable, was not changed on admixture with authentic methylmalonic acid. Final proof of identity was obtained by the infrared spectrum. Satisfactory analytical figures were difficult to obtain; after crystallisation from ethyl acetate–light petroleum a sample had m. p. 124—128° (decomp.) (Found: C, 37·6; H, 5·2. Calc. for $C_4H_6O_4,\frac{1}{2}H_2O$: C, 37·8; H, 5·5%).

Clemmensen Reduction of Rhodomyrtoxin.—Rhodomyrtoxin (0·1 g.) in ethanol (15 ml.) was refluxed for 8 hr. with amalgamated zinc (10 g.) and 6N-hydrochloric acid (12 ml.). The ether-soluble material was then isolated and treated overnight at room temperature with pyridine–acetic anhydride, to give the *tetra-acetate*, colourless needles (from ethanol), m. p. 172—174°, $\lambda_{max.}$ 232, 258, 284 mμ (ε 58,000, 15,000, 19,000) (Found: C, 68·1; H, 7·2. $C_{32}H_{40}O_9$ requires C, 67·7; H, 7·0%). The same material was obtained on reduction of rhodomyrtoxin in ethanol at 100°/80 atm. for 10 hr. in the presence of Raney nickel, followed by acetylation.

*Dide*iso*valerylrhodomrytoxin.*—A suspension of rhodomyrtoxin (1·1 g.) in hydriodic acid (30 ml.; *d* 1·7) was refluxed for 2 hr. and poured into water (250 ml.). The precipitate was filtered off, washed with water, and extracted with ether (5 × 100 ml.) The combined extracts were washed with sodium thiosulphate solution and with water, dried, and evaporated. The residue crystallised from ether–light petroleum, to give *dide*iso*valerylrhodomyrtoxin*, m. p. 300° (Found: C, 64·4; H, 5·2. $C_{14}H_{12}O_5$ requires C, 64·6; H, 4·6%). This gave in alkali a bright green solution which rapidly absorbed oxygen and faded to orange. With ferric chloride in ethanol it gave a red colour. Acetylation gave the *tetra-acetate*, m. p. (from butan-1-ol) 197—199°, $\lambda_{max.}$ 235, 275, 287 mμ (ε 36,300, 18,300, 18,000) (Found: C, 61·5; H, 4·85; Ac, 41·9%; *M*, 416. $C_{22}H_{20}O_9$ requires C, 61·7; H, 4·7; Ac, 40·5%; *M*, 428).

Tri-O-*methyldide*iso*valerylrhodomyrtoxin.*—Treatment of dide*iso*valerylrhodomyrtoxin, in methanol, with an excess of diazomethane, in ether, overnight at room temperature gave the *trimethyl ether*, m. p. (from ethanol) 171—172°, $\lambda_{max.}$ 235, 283, 290 mμ (ε 41,800, 24,200, 23,800) [Found: C, 67·3; H, 6·2; OMe, 29·6; *C*-Me, 9·2%; *M*, 300. $C_{12}H_3O_2Me_2(OMe)_3$ requires C, 67·6; H, 6·0; OMe, 30·7; *C*-Me, 9·9%; *M*, 302]. This gave a *trinitrobenzene adduct*, m. p. (from ethanol) 184—185° (Found: C, 53·6; H, 3·9; N, 8·0. $C_{17}H_{18}O_5,C_6H_3O_6N_3$ requires C, 53·6; H, 4·1; N, 8·15%), and a *picrate*, m. p. (from light petroleum) 185—186° (decomp.) (Found: N, 7·9. $C_{17}H_{18}O_5,C_6H_3O_7N_3$ requires N, 7·9%). Acetylation with pyridine–acetic anhydride in the usual way gave the *monoacetate*, m. p. (from butan-1-ol) 204—205° (Found: C, 66·2; H, 5·85. $C_{19}H_{20}O_6$ requires C, 66·3; H, 5·8%), while treatment with *p*-chlorobenzoyl chloride–pyridine gave the p-*chlorobenzoate*, m. p. (from butan-1-ol) 244—245° (Found: C, 65·5; H, 4·8; Cl, 8·5. $C_{24}H_{22}O_6Cl$ requires C, 65·2; H, 5·0; Cl, 8·1%).

Tetra-O-*methyldide*iso*valerylrhodomyrtoxin.*—A solution of the above trimethyl ether (0·3 g.) in acetone (40 ml.) and methyl iodide (10 ml.) was refluxed for 24 hr. during which potassium carbonate (4 g.) was gradually added in small portions. Isolation of the ether-soluble material and crystallisation from butan-1-ol gave the *tetramethyl ether*, m. p. 203—204°, $\lambda_{max.}$ 236, 272, 284, 296 mμ (ε 40,600, 18,200, 25,200, 22,800) [Found: C, 68·6; H, 6·2; OMe, 39·9. $C_{14}H_8O(OMe)_4$ requires C, 68·4; H, 6·4; OMe, 39·2%]. The *trinitrobenzene adduct*, crystallised from ethanol, had m. p. 234—235° (Found: N, 7·95. $C_{18}H_{20}O_5,C_6H_3O_6N_3$ requires N, 7·95%).

Action of Sodium in Liquid Ammonia on the Above Tetramethyl Ether.—The tetramethyl ether (0·2 g.) in tetrahydrofuran (20 ml.) was added to liquid ammonia (100 ml.), and sodium added to the resulting solution till a permanent blue colour was obtained. The ammonia was allowed to evaporate, and the residue divided into alkali-soluble and -insoluble fractions. The alkali-soluble material gave, on acetylation, a *diacetate*, m. p. (from butan-1-ol) 224°, $\lambda_{max.}$ 230, 262, 300 mμ (ε 38,300, 17,800, 22,000) [Found: C, 64·5; H, 5·45; OMe, 17·0. $C_{14}H_8O(OMe)_2(OAc)_2$ requires C, 64·5; H, 5·4; OMe, 16·7%], while methylation with potassium carbonate–methyl iodide re-formed the original tetramethyl ether. The alkali-insoluble fraction had $\lambda_{max.}$ 235, 282 mμ and was a mixture of several compounds which could not be separated.

Zinc-dust Distillation of Tetra-O-*acetyldide*iso*valerylrhodomyrtoxin.*—The tetra-acetate (4 g.) was thoroughly ground with zinc dust (300 g.), and the mixture, in 5 g. portions, heated to redness in Pyrex tubing (7 mm. bore) in a slow stream of nitrogen. The combined distillates, in light petroleum, were placed on a column of alumina (1 × 4 cm.) and the hydrocarbon fraction eluted with light petroleum–benzene (1 : 1). This fraction distilled at 100°/0·1 mm., and amounted to about 15 mg.

C-*Acylation of Dide*iso*valerylrhodomyrtoxin.*—The crude product obtained from refluxing rhodomyrtoxin (5 g.) with hydriodic acid (75 ml.), in acetic acid (50 ml.), was saturated with boron trifluoride without cooling and the resulting solution poured into water (500 ml.). After ½ hr., the solid was filtered off and recrystallised from acetic acid (200 ml.) in orange needles. This boron trifluoride adduct decomposed when dissolved in ethanol (50 ml.), to give *di*-C-*acetyldide*iso*valerylrhodomyrtoxin*, m. p. 300°, $\lambda_{max.}$ 235, 280, 293, 306, 390 mμ (ε 13,600, 21,000, 22,000, 17,100, 3800) (Found : C, 63·15; H, 4·35. $C_{18}H_{16}O_7$ requires C, 62·8; H, 4·65%).

iso*Rhodomyrtoxin.*—The crude product obtained from refluxing rhodomyrtoxin (5 g.) with hydriodic acid (75 ml.), in *iso*valeric acid (50 ml.), was saturated with boron trifluoride without cooling and the solution poured into water (1500 ml.). The sticky red precipitate was dissolved in ethanol (300 ml.), the solution heated on the steam-bath for 15 min., and poured into water (1500 ml.). Ether-extraction gave iso*rhodomyrtoxin*, m. p. (from acetic acid) 231—234°, $\lambda_{max.}$ 234, 280, 293, 307, 395 mμ (ε 23,600, 39,500, 38,500, 29,000, 4000) (Found : C, 67·5; H, 6·15. $C_{24}H_{28}O_7$ requires C, 67·3; H, 6·6%). Acetylation in the usual way gave the *tetra-acetate*, m. p. (from butan-1-ol) 190—191°, $\lambda_{max.}$ 254 mμ (ε 45,500) (Found : C, 64·35; H, 5·9. $C_{32}H_{36}O_{11}$ requires C, 64·4; H, 6·0%).

Extraction of Fresh Mature Fruit.—Fresh cherries were macerated and exhaustively extracted with acetone, and the combined extracts evaporated. Tests with mice showed the toxic material to be in the ether-soluble fraction. This (16 g.) in ether (100 ml.) was extracted with N-sodium carbonate (200 ml.), and the colourless aqueous layer discarded. Extraction of the ether with water (2 × 100 ml.) then gave a golden-orange extract which, after being washed with ether, was saturated with sodium sulphate, and the solution was shaken with ether (200 ml.). A dark slime (approx. 5 ml.) appeared at the solvent interface and was acidified and extracted with ether. Removal of the ether and crystallisation of the residue from aqueous methanol gave the toxic *product* as pale yellow needles (0·35 g.), m. p. 186—188°, $\lambda_{max.}$ 277, 280 mμ (ε 26,500, 33,400) (Found : C, 67·6; H, 6·45. $C_{24}H_{28}O_7$ requires C, 67·3; H, 6·6%). Warming this compound with 90% sulphuric acid gave *iso*valeric acid; with ferric chloride in ethanol it gave a dark blue colour.

Treatment with pyridine–acetic anhydride overnight at room temperature gave the *tetra-acetate*, m. p. (from ether–light petroleum) 117—119° (Found : C, 64·5; H, 6·1. $C_{32}H_{36}O_{11}$ requires C, 64·5; H, 6·05%). The *tetrabenzoate* had m. p. (from ethanol) 199—200° (Found : C, 74·5; H, 5·3. $C_{52}H_{44}O_{11}$ requires C, 74·0; H, 5·2%).

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