*Galloflavin. Part II.**

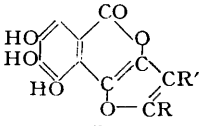
By JAMES GRIMSHAW, ROBERT D. HAWORTH, and (in part) H. K. PINDRED.

[Reprint Order No. 5874.]

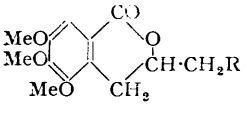
Structure (I; R = CO_2H, R′ = H; or *vice versa*) provisionally suggested for *iso*galloflavin has been confirmed by the oxidation of the hexahydro-decarboxylated derivative (II; R = CH_2·OH) of *iso*galloflavin to a carboxylic acid (II; R = CO_2H) identical with a synthetic specimen.

The mechanism of the formation of galloflavin from gallic acid is also discussed.

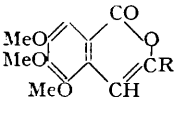
IN an earlier paper (Haworth and McLachlan, *J.*, 1952, 1583), *iso*galloflavin, for which structure (I; R = CO_2H, R′ = H; or *vice versa*) was tentatively suggested, has been shown to afford, on methylation and decarboxylation followed by catalytic reduction, tetra- and hexa-hydro-derivatives formulated as (IV) and (II; R = CH_2·OH) respectively. Further studies have now shown that the conversion of galloflavin into *iso*galloflavin by potassium hydroxide is almost quantitative when the reaction is carried out under nitrogen and that, with palladous oxide instead of palladium–charcoal as catalyst, the reduction proceeds rapidly and completely to the hexahydro-derivative (II; R = CH_2·OH).



(I)



(II)



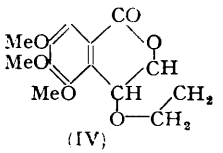
(III)

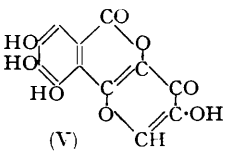
5 : 6 : 7-Trimethoxy*iso*coumarin-3-carboxylic acid (III; R = CO_2H) has been made readily available by complementary researches (Haworth, Pindred, and Jefferies, *J.*, 1954, 3617) and a synthesis of the hexahydro-derivative (II; R = CH_2·OH) was envisaged starting from this acid. The diazo-ketone (III; R = CO·CHN_2) described by Haworth, Pindred, and Jefferies (*loc. cit.*) readily gives the 3-alkoxyacetyl-5 : 6 : 7-trimethoxy*iso*-coumarin (III; R = CO·CH_2·OEt or CO·CH_2·O·CH_2Ph) on treatment with ethanol or

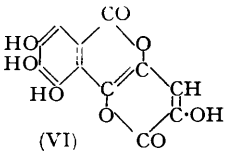
* Part I, *J.*, 1952, 1583.

benzyl alcohol and a boron trifluoride–ether catalyst according to the general procedure of Newman and Beal (*J. Amer. Chem. Soc.*, 1950, **72**, 5161). The benzyloxyacetyl derivative gave a crystalline ethylene mercaptal; the diethyl mercaptal was an unstable oil. Hydrogenolysis of either with active Raney nickel in boiling alcohol gave a compound, $C_{14}H_{16}O_5$, and not the expected ether (III; R = CH_2·CH_2·O·CH_2Ph). The ultra-violet absorption spectrum of the product was almost identical with that of 4-methyl-5 : 6 : 7-trimethoxy*iso*coumarin (Haworth, Pindred, and Jefferies, *loc. cit.*), suggesting that the compound was 3-ethyl-5 : 6 : 7-trimethoxy*iso*coumarin (III; R = Et), and the *iso*coumarin structure was confirmed by conversion into the corresponding *iso*carbostyril. The expected product (III; R = CH_2·CH_2·O·CH_2Ph), which is a masked β-alkoxy-ketone, may readily lose the elements of alcohol to give the easily reducible αβ-unsaturated ketone (cf. Heilmann, *Bull. Soc. chim.*, 1949, 66).

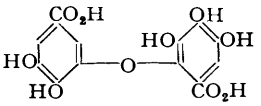
In view of the failure of this synthesis an attempt was made to oxidise the hexahydro-derivative (II; R = CH_2·OH) to the corresponding acid (II; R = CO_2H), a synthesis of which was envisaged by ascent from 5 : 6 : 7-trimethoxy*iso*coumarin-3-carboxylic acid (III; R = CO_2H) and subsequent reduction. Oxidation of the hexahydro-derivative (II; R = CH_2·OH) in dilute acetic acid solution with potassium dichromate and sulphuric acid gave 50% yields of the corresponding acid (III; R = CO_2H) which was obtained in dimorphous modifications, each affording the same methyl ester on methylation with diazomethane. Chain extension of 5 : 6 : 7-trimethoxy*iso*coumarin-3-carboxylic acid (III; R = CO_2H) had previously (*idem, loc. cit.*) been effected in poor yield by the classical Wolff rearrangement of the diazo-ketone (III; R = CO·CHN_2) to give methyl 5 : 6 : 7-trimethoxy*iso*coumarinyl-3-acetate (III; R = CH_2·CO_2Me), but application of Newman and Beal's conditions (*J. Amer. Chem. Soc.*, 1950, **72**, 5163) gave a marked improvement in yields. Here *tert.*-butanol, with a silver benzoate catalyst in triethylamine solution, gave the *tert.*-butyl ester (III; R = CH_2·CO_2Bu^t) which on acid hydrolysis afforded an amorphous acid, esterified with diazomethane to give the methyl ester (III; R = CH_2·CO_2Me) previously reported (Haworth, Pindred, and Jefferies, *loc. cit.*). Catalytic hydrogenation of the *tert.*-butyl ester proceeded smoothly and the oily product on acid hydrolysis gave the diamorphous 3 : 4-dihydro-5 : 6 : 7-trimethoxy*iso*coumarin-3-ylacetic acid (II; R = CO_2H), identical in all respects with the oxidation product of hexahydro-decarboxylated trimethyl*iso*-galloflavin.

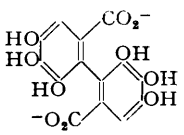

(IV)

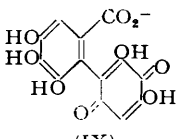

(V)


(VI)

In Part I (*loc. cit.*) formulæ (V) and (VI) were tentatively proposed for galloflavin, and (VI) is now preferred because formation of this structure from gallic acid can be presented by a rational mechanism whereas the open-chain intermediates in the formation of (V) would be unstable to alkali. The oxidation of pyrogallol derivatives is very dependent on conditions. For example, in a phosphate buffer pyrogallol is autoxidised to purpurogallin (Loew, *J. pr. Chem.*, 1877, **15**, 322) with hydroxy-*o*-quinones as intermediate products

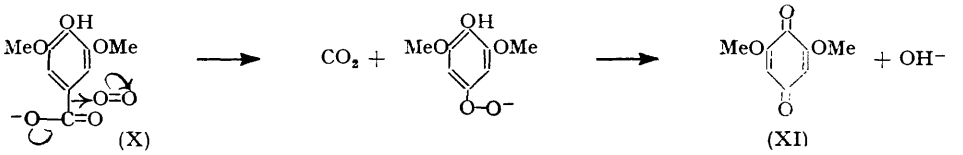

(VII)


(VIII)


(IX)

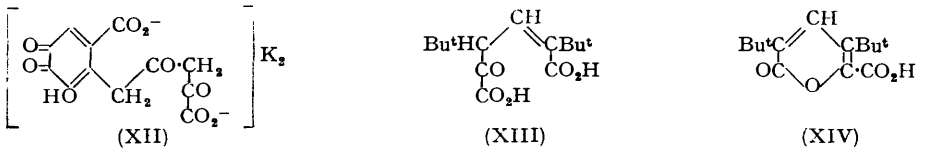
(Critchlow, Haworth, and Pauson, *J.*, 1951, 1319), but in strongly alkaline solution pyrogallol and ethyl gallate yield 2 : 3 : 4 : 2′ : 3′ : 4′-hexahydroxydiphenyl (Harries, *Ber.*, 1902, **35**, 2957) and ellagic acid (Herzig, Tscherne, and Bronneck, *Monatsh.*, 1908, **29**, 277) respectively, presumably by symmetrical dimerisation of semiquinone radicals;

unsymmetrical union of two gallic acid semiquinone radicals will account for the biogenesis of dehydrodigallic acid (VII), isolated by Schmidt and Mayer (*Annalen*, 1952, **578**, 34) from the tannin of Spanish chestnut. When a potassium hydroxide solution of gallic acid is aerated the anion (VIII) is probably produced and converted by further oxidation into (IX), and in support of this stage it has been found that syringic acid (X) is easily oxidised by air to 2 : 6-dimethoxy-*p*-benzoquinone (XI). Structure (IX) may be



(X) (XI)

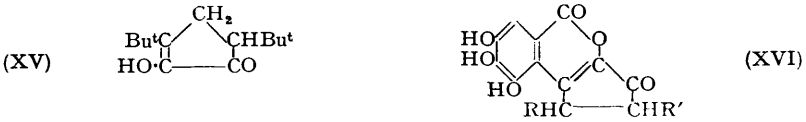
represented in equivalent α- and β-diketone forms and oxidation, hydrolysis, and decarboxylation reactions provide several equally attractive representations of ring rupture leading to the " dipotassium salt " (XII); subsequent conversion of (XII) into galloflavin (VI) presents no difficulty. The " dipotassium salt " precipitated during the preparation



(XII) (XIII) (XIV)

of galloflavin gives an aqueous solution of pH 7—8 and consequently affords carboxylate and not phenoxide ions. It is not a simple salt of galloflavin because whilst the latter may be converted into *iso*galloflavin (I; R = CO_2H, R′ = H) in high yield, the " dipotassium salt " under similar conditions gives an amorphous product which on methylation with diazomethane gives traces only of tetramethyl*iso*galloflavin; the main product is amorphous and insoluble in methanol and contains only 2% of methoxyl.

Support for these views also comes from recent work of Campbell *et al.* (*J. Amer. Chem. Soc.*, 1951, **73**, 4190) on the oxidation of 4 : 6-di-*tert.*-butylpyrogallol. The products (XIII) and (XIV) arise by reaction mechanisms similar to those proposed above for galloflavin, and the third product (XV) arises by ring contraction of the hydroxy-*o*-quinone intermediate. An analogous change from the galloflavin intermediate ion (VIII) would



(XV) (XVI)

lead to the acid (XVI; R = CO_2H, R′ = H) which, as the vinylogue of a β-keto-acid, may equally well account for the properties of brevifolincarboxylic acid recently isolated from *Algarobilla* by Schmidt and Bernauer (*Annalen*, 1954, **288**, 211) and to which these authors assign structure (XVI; R = H, R′ = CO_2H).

Experimental

3-Ethoxyacetyl-5 : 6 : 7-trimethoxyisocoumarin (III; R = $CO{\cdot}CH_2{\cdot}OEt$).—3-Diazoacetyl-5 : 6 : 7-trimethoxy*iso*coumarin (Haworth, Pindred, and Jefferies, *loc. cit.*) (2 g.) was suspended in ethanol (10 c.c.) and treated with boron trifluoride–ether complex (1 g.). When evolution of nitrogen had ceased, the pale yellow solution was refluxed for 10 min. and cooled; the *α-ethoxyketone* separated and crystallised from ethanol as colourless needles (2 g.), m. p. 122—123° (Found: C, 59·5; H, 5·6. $C_{16}H_{18}O_7$ requires C, 59·6; H, 5·6%). The 2 : *4-dinitrophenylhydrazone* separated from glacial acetic acid in orange needles, m. p. 236—237° (Found: C, 52·9; H, 4·3; N, 11·4. $C_{22}H_{22}O_{10}N_4$ requires C, 52·6; H, 4·4; N, 11·2%).

3-Benzyloxyacetyl-5 : 6 : 7-trimethoxyisocoumarin (III; R = $CO{\cdot}CH_2{\cdot}O{\cdot}CH_2Ph$).—3-Diazoacetyl-5 : 6 : 7-trimethoxy*iso*coumarin (2 g.) was suspended in benzyl alcohol (6 c.c.) and

treated with boron trifluoride–ether complex (1 g.). When evolution of nitrogen had ceased, the mixture was warmed on a water-bath for 10 min., diluted with an equal volume of methanol, and set aside at 0°. The *α-benzyloxy-ketone* separated and crystallised from a large volume of ethanol as colourless plates (2·2 g.), m. p. 145—146° (Found : C, 65·4; H, 5·3. $C_{21}H_{20}O_7$ requires C, 65·7; H, 5·2%). The 2 : 4-*dinitrophenylhydrazone* crystallised from glacial acetic acid as orange needles, m. p. 210—211° (Found : N, 9·85. $C_{27}H_{24}O_{10}N_4$ requires N, 9·9%).

3-*Benzyloxyacetyl*-5 : 6 : 7-*trimethoxy*isocoumarin *Diethyl Mercaptal.*—Anhydrous sodium sulphate (1·5 g.) was added to a solution of the above α-benzyloxy-ketone (0·5 g.) and zinc chloride (1·5 g.) in ethanethiol (30 c.c.). When the mixture had stood for 18 hr. in ice, excess of ethanethiol was removed under reduced pressure and the residue shaken with 0·5N-hydrochloric acid and ether. The ethereal layer was washed with water and dried (Na_2SO_4) and the solvent removed under reduced pressure. The residual pale yellow pungent oily diethyl mercaptal, which was used as such in subsequent work, was readily decomposed by heat and gave a precipitate with alcoholic mercuric chloride.

3-*Benzyloxyacetyl*-5 : 6 : 7-*trimethoxy*isocoumarin *Ethylene Mercaptal.*—Dry hydrogen chloride was passed through a suspension of anhydrous sodium sulphate (1·5 g.) and the above α-benzyloxy-ketone (III; R = $CO{\cdot}CH_2{\cdot}O{\cdot}CH_2Ph$) (0·5 g.) in a mixture of dioxan (10 c.c.) and ethanedithiol (0·15 c.c.). After 3 hr. the supply of hydrogen chloride was discontinued and the mixture, now bright red, was set aside overnight, then poured into water and extracted with ether. The ethereal extract was washed with water and dried (Na_2SO_4) and the solvent removed; the residual *ethylene mercaptal* crystallised from ethanol as a mat of fine needles, m. p. 133—134° (Found : C, 60·3; H, 5·2; S, 14·1. $C_{23}H_{24}O_6S_2$ requires C, 60·0; H, 5·2; S, 13·9%).

3-*Ethyl*-5 : 6 : 7-*trimethoxy*isocoumarin (III; R = Et).—A solution of either mercaptal (1·0 g.) in ethanol (100 c.c.) was refluxed with Raney nickel (12 g.) for 18 hr. The solvent was decanted and the nickel washed successively with ethanol (3 × 100 c.c.) and acetone (5 × 100 c.c.). These washings and the reaction solution were combined and centrifuged, and the solvent was removed, leaving a pale yellow residue (0·3 g.) which was dissolved in benzene (25 c.c.) and chromatographed on alumina (10 g.). Elution with benzene yielded, on evaporation, colourless crystals (0·2 g.) of 3-*ethyl*-5 : 6 : 7-*trimethoxy*isocoumarin (III; R = Et) which recrystallised from benzene–light petroleum (b. p. 60—80°) in needles, m. p. 69—70° (Found : C, 64·0; H, 5·9. $C_{14}H_{17}O_5$ requires C, 63·7; H, 6·1%). No further crystalline material could be eluted with benzene, ether, or acetone.

The 3-ethyl*iso*coumarin, m. p. 69—70° (0·08 g.), was heated with methanolic ammonia (3 c.c.) in a sealed tube at 120° for 3 hr. The solution was evaporated to dryness, yielding 3-*ethyl*-5 : 6 : 7-*trimethoxy*isocarbostyril as a white solid (0·07 g.) which crystallised from methanol in long colourless needles, m. p. 171—171·5° (Found : N, 4·8. $C_{14}H_{17}O_4N$ requires N, 5·3%).

3 : 4-*Dihydro*-3-2′-*hydroxyethyl*-5 : 6 : 7-*trimethoxy*isocoumarin (II; R = $CH_2{\cdot}OH$).—Decarboxylated trimethyl*iso*galloflavin (Haworth and McLachlan, *loc. cit.*) (0·25 g.) was dissolved in 90% acetic acid (30 c.c.) and shaken with palladous oxide (0·02 g.) in hydrogen at 50° and atmospheric pressure. Hydrogen uptake (65 c.c.; calc., 64 c.c.) was complete in 2 hr. The catalyst and solvent were removed, and the hexahydro-derivative crystallised from benzene–light petroleum (b. p. 60—80°) in needles (0·15 g.), m. p. 83—84° undepressed on admixture with an authentic specimen but depressed to 70—75° on admixture with the tetrahydro-derivative (IV), m. p. 82—83°.

tert.-*Butyl* 5 : 6 : 7-*Trimethoxy*isocoumarin-3-*ylacetate* (III; R = $CH_2{\cdot}CO_2Bu^t$).—A freshly filtered solution of silver benzoate (1 g.) in triethylamine (7 c.c.) was added portionwise during 1½ hr. to a boiling solution of 3-diazoacetyl-5 : 6 : 7-trimethoxy*iso*coumarin (2 g.) in dry *tert.*-butanol (50 c.c.). Refluxing was continued for a further ½ hr.; then the excess of silver salts was decomposed with a little formic acid, and the mixture treated with charcoal, filtered, and evaporated to a brown resin. The resin was chomatographed in benzene (25 c.c.) on alumina (50 g.). Elution with benzene yielded, on evaporation, colourless crystals closely followed by a red oil. Recrystallisation of the first eluant from *cyclo*hexane afforded tert.-*butyl* 5 : 6 : 7-*trimethoxy*isocoumarin-3-*ylacetate* (1·0 g.) as stout needles, m. p. 100—101° (Found : C, 61·2; H, 6·4. $C_{18}H_{22}O_7$ requires C, 61·7; H, 6·3%).

Methyl 5 : 6 : 7-*Trimethoxy*isocoumarinyl-3-*acetate* (III; R = $CH_2{\cdot}CO_2Me$).—The above *tert.*-butyl ester (0·12 g.) was dissolved in a mixture of acetic acid (3 c.c.), concentrated hydrochloric acid (1 c.c.), and water (1 c.c.), and the solution was refluxed for 2 hr. The resulting dark brown solution was diluted with water and extracted with ether. The ethereal extract was washed with water and sodium hydrogen carbonate solution. Acidification of the

carbonate extract gave an amorphous acid (0·03 g.). This acid, isolated with ether, was dissolved in methanol and methylated with excess of ethereal diazomethane. The methyl ester crystallised from benzene–light petroleum (b. p. 60—80°) as prisms, m. p. 127—128° (Haworth, Pindred, and Jefferies, *loc. cit.*, give m. p. 127—128°).

3 : 4-*Dihydro*-5 : 6 : 7-*trimethoxy*isocoumarin-3-*ylacetic Acid* (II; R = CO_2H).—(*a*) The above *tert.*-butyl ester (0·84 g.) was shaken in glacial acetic acid (25 c.c.) with 25% palladium–charcoal in an atmosphere of hydrogen at 50° and atmospheric pressure. Hydrogen uptake (57 c.c.; calc., 57 c.c.) was complete after 12 hr. Removal of the catalyst and solvent gave a viscous syrup which was hydrolysed by 2 hours' refluxing with acetic acid (15 c.c.), concentrated hydrochloric acid (5 c.c.), and water (5 c.c.). Evaporation of the solvent under reduced pressure gave 3 : 4-*dihydro*-5 : 6 : 7-*trimethoxy*isocoumarin-3-*ylacetic acid* as a pale yellow solid, crystallising from 5% acetic acid solution in colourless rhombs (0·6 g.), m. p. 155—156° [Found: C, 57·1; H, 5·6%; equiv., 294 (cold), 147·5 (hot). $C_{14}H_{16}O_7$ requires C, 56·8; H, 5·4%; equiv., 296 (cold), 148 (hot)].

(*b*) 3 : 4-Dihydro-3-2′-hydroxyethyl-5 : 6 : 7-trimethoxy*iso*coumarin (II; R = CH_2·OH) (85 mg.) was dissolved in a mixture of acetic acid (1 c.c.), water (3 c.c.), and concentrated sulphuric acid (0·5 c.c.). A solution of potassium dichromate (70 mg.) in a little 10% acetic acid was added dropwise during 2 hr. to the cooled solution. The mixture was then warmed on a water-bath for 30 min. to complete the reaction, diluted with water, and continuously extracted with ether for 3 hr. The ethereal extract was evaporated to dryness, and the residue redissolved in ether, and extracted with sodium hydrogen carbonate solution. Acidification of the carbonate extract followed by extraction with ether and evaporation of the solvent left the *acid* (45 mg.) which crystallised from dilute acetic acid (5%) in small rhombs, m. p. 155—156° (Found: C, 56·7; H, 5·8), undepressed on admixture with 3 : 4-dihydro-5 : 6 : 7-trimethoxy*iso*coumarinyl-3-acetic acid (II; R = CO_2H) obtained by method (*a*).

Prepared by either method, the acid when crystallised from its cold supersaturated dilute acetic acid solutions afforded an unstable dimorph as plates, m. p. 140—141° (with resolidification and m. p. 155—156°). Methylation of either form in methanol with ethereal diazomethane and evaporation of the solvents gave the *methyl ester* which crystallised from benzene–light petroleum (b. p. 60—80°) as small colourless rhombs, m. p. 109—110° [Found: from method (*a*), C, 57·9; H, 6·0; from method (*b*), C, 58·2; H, 5·8. $C_{15}H_{18}O_8$ requires C, 58·1; H, 5·8%]. The m. p. of the methyl ester and both forms of the synthetic acid (method *a*) were not depressed on admixture with the corresponding compounds derived by oxidation of the hexahydro-derivative (II; R = CH_2·OH) (method *b*). The m. p.s of the higher-melting forms of both synthetic and degradation acid were not depressed on admixture with the lower-melting forms of both synthetic and degradation material.

Autoxidation of Syringic Acid.—Syringic acid (0·5 g.), potassium hydroxide (0·15 g.), and disodium hydrogen phosphate (5 g.) were dissolved in water (50 c.c.), and air was blown through the solution at room temperature for 4 days, the mixture being extracted at frequent intervals with chloroform. The chloroform extracts were combined and dried ($NaSO_4$) and the solvent was evaporated, leaving a yellow solid (0·02 g.) crystallising from dilute acetic acid as needles, m. p. 250—251° undepressed on admixture with an authentic sample of 2 : 6-dimethoxy-*p*-benzoquinone (XI). Acidification of the phosphate solution precipitated syringic acid (0·21 g.) which crystallised from water as needles, m. p. 204—205°.

Action of Alkali on "Galloflavin Dipotassium Salt" (XII).—The "dipotassium salt" intermediate in the preparation of galloflavin (2 g.) was dissolved in 10% potassium hydroxide solution (36 c.c.) under nitrogen. After 45 min., the solution was acidified with hydrochloric acid and boiled for a short time. The resulting chocolate-brown precipitate (0·65 g.) was treated overnight with excess of ethereal diazomethane, the solvent removed, and the residue extracted twice with boiling methanol (20 c.c.), leaving a light brown amorphous residue (0·5 g.) (Found: OMe, 2·0%). Tetramethyl*iso*galloflavin (0·06 g.), recovered from the methanol extracts, had m. p. 222—224°, raised to 227—228° by crystallisation from methanol and gave no depression in m. p. on admixture with an authentic specimen, m. p. 232—234°.

Our thanks are offered to the Department of Scientific and Industrial Research for a Maintenance Allowance (to J. G.), and to Imperial Chemical Industries Limited for a grant towards the costs of the investigation.

THE UNIVERSITY, SHEFFIELD, 10. [*Received, November 12th*, 1954.