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SOME PHENOLIC CONSTITUENTS OF *GENTIANA LUTEA*

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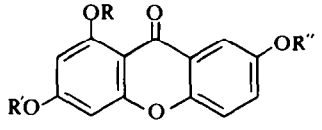
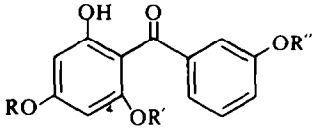
Abstract—Gentisein, gentisin, iso-gentisin, 1-hydroxy-3,7-dimethoxyxanthone, 1,3,7-trimethoxyxanthone and 2,3',4,6-tetrahydroxybenzophenone have been isolated from *Gentiana lutea*. Their inter-relationship is discussed.

Gentiana lutea, which is still used in Pharmacy for its bitter principles, was the first plant source reported to contain a xanthone. Gentisin so isolated in 1821[1] was subsequently described as a monomethyl ether of 1,3,7-trihydroxyxanthone (Gentisein) which Kostenecki[2] and later Perkin[3] suggested was 1,7-dihydroxy-3-methoxyxanthone (I; R = R'' = H, R' = Me). This suggestion was substantiated by synthesis in 1947.[4] In 1955 Cannonica and Pelizzoni isolated iso-gentisin and established its structure as 1,3-dihydroxy-7-methoxyxanthone (I; R = R' = H, R'' = Me).[5] We have re-examined the dried root of *G. lutea* and confirm these previous observations; however, in the fresh rhizome four further phenolic compounds have been isolated and characterized.

The extract from fresh *G. lutea* rhizome was separated into a phenolic and neutral fraction and each fraction further separated on preparative TLC. The phenolic fraction yielded 1,3,7-trihydroxyxanthone[6] (I; R = R' = R'' = H; 0·002%) with



I



II

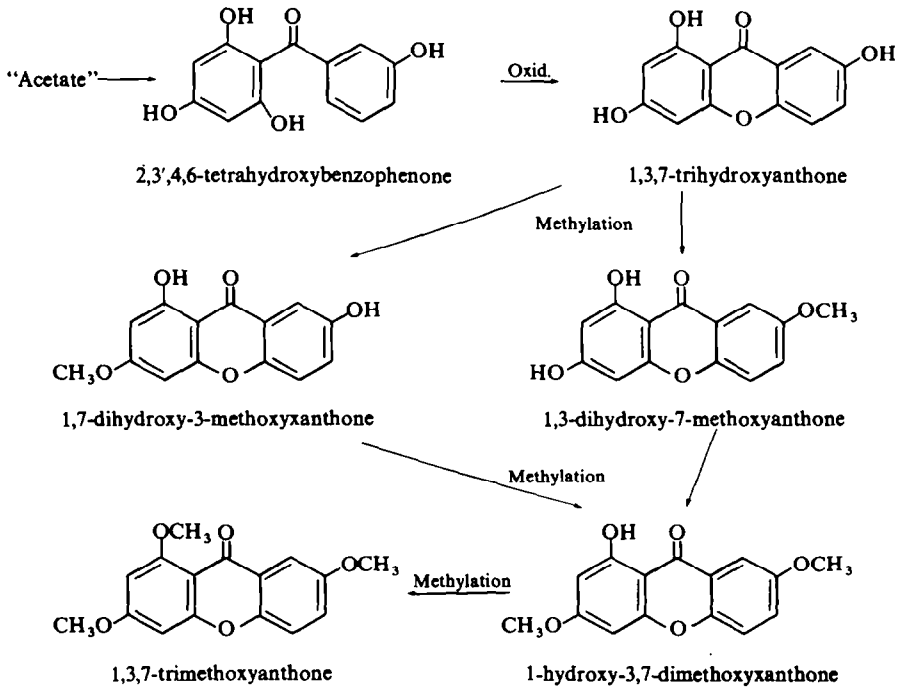
R_f = 0·36 on silica gel using benzene–ethyl acetate (3:1) as eluant. At R_f = 0·58–0·60 a mixture of 1,3-dihydroxy-7-methoxyxanthone (I; R = R' = H, R'' = Me) and 1,7-dihydroxy-3-methoxyxanthone (I; R = R'' = H, R' = Me) was isolated (0·014%). The neutral fraction yielded 1-hydroxy-3,7-dimethoxyxanthone (I; R = H, R' = R'' = Me) and 1,3,7-trimethoxyxanthone (I; R = R' = R'' = Me) with R_f = 0·82 and 0·39, the yield being 0·01% and 0·002%, respectively, of the rhizome extracted.

In view of the possible inter-relationship between hydroxybenzophenones and xanthones suggested in 1963[7] and the establishment of oxidative coupling as a ready means for the synthesis of xanthones under "physiological type" reaction conditions[8–10] together with the synthesis of 1,3,7-trihydroxyxanthone (Gentisein; I; R = R' = R'' = H) from 2,3',4,6-tetrahydroxybenzophenone (II; R = R' = R'' =

H) by oxidation,[11] a careful examination of the extract of *G. lutea* was made to establish whether 2,3',4,6-tetrahydroxybenzophenone, the implied precursor to Gentisein, was present.

Extraction of material from the TL chromatograms of the phenolic fraction at R_f = 0·07 to 0·11, i.e. within the R_f range found for synthetic benzophenone, gave an uncharacterizable oil (98 mg). When this oil was methylated and examined by gas chromatography a peak with a retention volume of 9·75 litres, corresponding to 2,3',4-trimethoxy-6-hydroxybenzophenone (II; R.=.R' = R'' = Me), was observed. Quantitative comparisons between naturally occurring and synthetic benzophenone established the concentration of 2,3',4,6-tetrahydroxybenzophenone (II; R = R' = R'' = H) in the fresh rhizome of *Gentiana lutea* as 0·0003%.

A confirmation to this gas chromatographic result was established by ^{14}C radio tracer dilution analysis. In our studies on the biosynthesis of xanthones produced by *G. lutea*, we have established that rapid assimilation of sodium acetate-2-^{14}C occurs under tissue culture conditions. In a typical experiment 0·1 mc of sodium acetate was incubated for 3 days by slow aeration of *G. lutea* in water at pH 6.4 and an incorporation of radioactivity of 0·53% was obtained, based on the xanthone fraction (gentisein, gentisin and iso-gentisin) isolated. Extraction of the TL chromatograms corresponding to where 2,3',4,6-tetrahydroxybenzophenone (II; R = R' = R'' = H) should be located and dilution of this extract with inactive benzophenone followed by purification gave 2,3',4,6-tetrahydroxybenzophenone with an activity of 449 dpm/mg. This corresponds to a total activity for the naturally occurring benzophenone of 22,450 dpm. The mixed xanthones, isolated from the rhizome, were demethylated and the gentisein so produced purified and counted to give an activity



of 20,650 dpm/mg. If we consider the inter-relationship between benzophenone and Gentisein as involving solely an oxidative coupling with loss of two hydrogen atoms, then the activity of Gentisein should be almost equivalent (244/246) to the activity of the benzophenone. Using this assumption and basing the methylation of rhizome extract at 50%, as found during the synthetic benzophenone methylative procedures, the radiochemical dilution analysis gives the concentration of 2,3',4,6-tetrahydroxybenzophenone (II; R = R' = R'' = H) in fresh *Gentiana lutea* as 0·0005%.

These results support the suggestion that one biosynthetic route to xanthones is through oxidative coupling[7] and Scheme 1 indicates the inter-relationship between the compounds found in *Gentiana lutea*. Hither to this publication, the only co-occurrence of benzophenone and xanthone was reported in extracts of *Symphonia globulifera* L.[12]

EXPERIMENTAL

UV spectra were measured in ethanol, IR spectra as nujol films and the NMR spectra were recorded in trifluoroacetic acid or deuterochloroform† as indicated. Gas chromatographic data was obtained on a Perkin Elmer F 11 Chromatograph using their DE 301 glass column. Liquid scintillation counting was carried out using Nuclear Enterprises NE 220 liquid scintillator (dioxan based) in a Nuclear Chicago Scintillation Counter Model 6822, quench corrections being obtained by the channels ratio method.

1.3.7-*Trihydroxyxanthone* (*gentisein*, I; R = R' = R'' = H)

This xanthone was synthesized by the condensation of phoroglucinol with 2,5-dihydroxybenzoic acid[6] to give a solid crystallizing from EtOAc with m.p. 315–319° d, Lit. m.p. 316–318°[6]; R_f = 0·36 (silica gel, benzene/EtOAc 3:1); λ_{max} 220 sh., 238, 260, 310, 373 mμ log ε = 4·22, 4·47, 4·55, 4·18, 3·82; ν_{max} (CO) 1620, 1665, (OH) 3370 cm^{-1}. Insolubility prevented a satisfactory NMR spectrum from being obtained.

1,3-*Dihydroxy-7-methoxyxanthone* (*isogentisin*; I; R = R' = H, R'' = Me)

The condensation of phloroglucinol with 2-hydroxy-5-methoxybenzoic acid[6] gave isogentisin, m.p. 245–246°, Lit. m.p. 239–240°[6]; R_f = 0·593 (silica gel, benzene/EtOAc 3:1); λ_{max} 208 sh., 236, 259, 311, 370 mμ log ε = 4·05, 4·45, 4·53, 4·11, 3·77; ν_{max} (CO) 1630, 1650, (OH) 3520 cm^{-1}; (1—OH) −1·56 τ, (3—OH) −0·22 τ, (7—OMe) 5·90 τ.

1,7-*Dihydroxy-3-methoxyxanthone* (gentisin; I; R = H, R' = Me)

Selective methylation of Gentisein gave 1,7-dihydroxy-3-methoxyxanthone, m.p. 273–275°, Lit. m.p. 267°[5]; R_f = 0·589 (silica gel, benzene/EtOAc 3:1); λ_{max} 205, 237·5, 307, 375 mμ log ε = 3·91, 4·31, 4·43, 3·99, 3·63; ν_{max} (CO) 1610, 1660, (OH) 3400 cm^{-1}; (1—OH) −1·37 τ, (7—OH) −0·48 τ, (3—OMe) 5·93 τ.

1-*Hydroxy*-3,7-*dimethoxyxanthone* (I; R = H, R' = R'' = CH_3)

Gentisein (0·6 g) was refluxed with Me_2SO_4 (0·24 ml) in dry acetone (40 ml) containing anhyd K_2CO_3 (1 g) for 18 hr. After filtration and evaporation the residue was crystallized from MeOH to give a solid (0·44 g), m.p. 168–169·5°, Lit. m.p. 167°[5]; R_f = 0·82 (silica gel, benzene/EtOAc 3:1); λ_{max} 207, 236, 259, 308, 370 mμ log ε = 3·20, 3·48, 3·61, 3·16, 2·73; ν_{max} (CO) 1620, 1660, (OH) 3420 cm^{-1}; (1—OH) −2·90 τ, (3 and 7—OMe) 6·09 τ.†

1,3,7-*Trimethoxyxanthone* (I; R = R' = R'' = Me)

3,7-Dimethoxy-1-hydroxyxanthone (0·2 g) was completely methylated with Me_2SO_4 (1·0 ml) in acetone–K_2CO_3 by reflux for 3 hr, to yield, after the usual work.

1,3,7-trimethoxyxanthone (198 mg) which crystallized from EtOAc, m.p. 173–174°, Lit. m.p. 171–173°[6]; R_f = 0·39 (silica gel, benzene/EtOAc 3:1); λ_{max} 207, 239 sh., 255, 303, 356 mμ log ε = 3·20, 3·47, 3·60, 3·12, 2·82; ν_{max} (CO) 1625, 1647 cm^{-1}; (1—OMe) 6·06 τ, (3 and 7—OMe) 6·13 τ.†

2,3',4,6-*Tetrahydroxybenzophenone* (II; R = R' = R'' = H)

Dimethylation of 2,4,6-trihydroxy-3'-methoxybenzophenone[11] gave 2,3',4,6-tetrahydroxybenzophenone,

m.p. 235–238°; R_f = 0·1 (silica gel, benzene/EtOAc 3:1); λ_{max} 210, 262, 308 mμ log ε = 4·50, 3·65, 4·25; ν_{max} (CO) 1638, (OH) 3250, 3350 cm^{-1}; (2,6-OH) −0·45 τ, (3'-OH) 0·73 τ, (4-OH) 1·59 τ (deutero-acetone).

2,3',4-*Trimethoxy-6-hydroxybenzophenone* (II; R = R' = R'' = Me)

2,4,6-Trihydroxy-3'-methoxybenzophenone (0·5 g) was methylated with Me_2SO_4 (5 ml) in acetone–K_2CO_3 under reflux for 6 hr. After work up in the usual way a neutral fraction (253 mg) was obtained which crystallized from benzene 2,3',4-*trimethoxy-6-hydroxybenzophenone*, m.p. 123–125°, b.p. 180–188°/0·1 mm Hg. (Found: C, 67·3, H, 6·0, $C_{16}H_{16}O_5$ requires: C, 66·7; H, 5·6%); λ_{max} 209, 221 sh., 254, 310 mμ log ε = 4·32, 4·05, 3·61, 3·27; ν_{max} (CO) 1648 cm^{-1}; (2-OH) −2·87 τ, (3'-Me) 6·12 τ, (4-OMe) 6·18 τ, (6-OMe) 6·23 τ.† A repeated methylation gave the same quantity of trimethyl ether (~50%) as did methylation of 2,3',4,6-tetrahydroxybenzophenone under the same conditions.

Extraction of Gentiana lutea

(a) *Dried root.* The dried root (50 g) was extracted with MeOH (500 ml) for 5 days in a Soxhlet apparatus. Evaporation of the MeOH left a brown oil (20 g) which was hydrolysed with dil HCl (62·5 ml, 3%) by heating in a steam bath for 1 hr. Extraction of this soln with EtOAc (500 ml) followed by washing with $NaHCO_3$ aq (250 ml, 5%), water, drying with anhyd. $MgSO_4$, filtration and evaporation left a semi-solid residue (0·644 g). This solid was separated on preparative TLC using silica gel and elution with benzene–EtOAc (3:1) to give a band at R_f = 0·58–0·60 which on extraction with MeOH gave a solid (0·3 g, 0·6%). This solid showed all the characteristics of a mixture of gentisin and iso-gentisin. R_f = 0·589 and 0·593, respectively, on TLC. Demethylation of this mixture (100 mg) by reflux in AcOH (10 ml) with HBr (3·5 ml, 60%) for 7 hr gave, after pouring into water and extracting with EtOAc (50 ml), washing with water and $NaHCO_3$ aq, drying and evaporation, a solid (81 mg) which separated on TLC at R_f = 0·36 into 1,3,7-trihydroxyxanthone (69 mg, 70%) (I; R = R' = R'' = H), m.p. and m. m.p. 300–303° d. and at R_f = 0·59 to give 1,7-dihydroxy-3-methoxyxanthone (gentisin; 12 mg, 12%; I; R = R'' = H, R' = Me), m.p. and m.m.p. 272–275°.

(b) *Fresh root.* Fresh *Gentiana lutea* rhizome (4782 g) was pulverized in a blender with MeOH and then extracted (Soxhlet) with more MeOH (15 l.) for 5 days. After evaporation of the MeOH the residue was dissolved in EtOAc (1500 ml) and the EtOAc layer washed with $NaHCO_3$ aq (500 ml, 10%), NaOH aq (9 l., 10%), water, dried with anhyd $MgSO_4$, filtered and evaporated to dryness to give the *neutral* fraction (40·1 g). The NaOH soln was acidified and extracted with EtOAc (500 ml) which, after washing, drying and evaporation, gave the *phenolic* fraction (14·8 g).

The phenolic fraction was triturated with benzene to give a solid (10·9 g) and an oily residue (3·9 g). A portion of the solid (1·5 g) was separated on preparative TLC using silica gel with elution by benzene–EtOAc (3:1) to give:

(a) A band at R_f = 0·36 which on extraction with MeOH and evaporation gave a solid (98 mg) which on re-chromatography using silica gel with benzene–EtOAc (3:1) gave 1,3,7-trihydroxyxanthone (I; R = R' = R'' = H) (10 mg, 0·002% of rhizome extracted), m.p. and m.m.p. 297–300° d.

(b) A band at R_f = 0·58–0·60 yielded a mixture of gentisin and iso-gentisin (87 mg, 0·014%) similar to that obtained from the dried root extract.

(c) A band at R_f = 0·1 corresponding to 2,3',4,6-tetrahydroxybenzophenone was removed (10 mg) but no pure compound was isolated from this extract.

The oily residue from the trituration showed no bands corresponding to xanthones or benzophenone and was not examined further.

The neutral fraction (10·2 g) was distilled and a fraction boiling between 160–188°/0·1 mmHg collected (1·35 g). Separation of this distillate on TLC (silica gel, benzene–EtOAc 3:1) gave a band at (a) R_f = 0·82 which on extraction from the absorbent and evaporation gave a solid (117 mg) which proved to be identical with I (R = H, R' = R'' = Me) by comparison of m.p., UV and IR spectra; yield 0·096%. (b) R_f = 0·39 which yielded a solid (22 mg) having identical UV and IR spectra to I (R = R' = R'' = Me), m.p. and m. m.p. 172–175°; yield = 0·0018% of rhizome extracted.

Characterization of 2,3',4,6-*tetrahydroxybenzophenone* (II; R = R' = R'' = H) *in* G. lutea.

(a) *Gas chromatography.* The ppt from the phenolic fraction of the fresh rhizome extract (10·9 g) was separated on TLC (silica gel, benzene–EtOAc 3:1) to give a band at R_f = 0·1 (corresponding to 2,3',4,6-tetrahydroxybenzophenone) which on extraction with MeOH, filtration and evaporation, yielded an oil (98 mg). Since this oil could not be characterized a portion was methylated (32 mg), as described previously

for benzophenone methylation, to give a neutral fraction (12 mg). Gas chromatographic analysis using a SE 30 silicone gum rubber liquid phase column at 230°, gave a peak with a retention volume of 9·75 litres. This peak was identical to a peak obtained from II (R = R′ = R″ = H) under the same conditions. Quantitative comparisons gave the concentration of benzophenone as 2·27 mg. The concentration of benzophenone in the original extract (allowing for 50% conversion to methyl ether) becomes 13·9 mg, i.e. 0·0003%. Benzophenone could not be detected in the neutral fraction extract of fresh root or in the extract from the dried root.

(b) *Radio-active dilution analysis.* The rhizome from a *G. lutea* plant (215 g) was cut into slices and washed with water for 4 hr. Water (tap, 400 ml) containing 1 drop of dil HCl (to give a pH of 6 to 6·5) NaOAc-2-^{14}C (0·1 mc, 0·22 mg) was added and incubation allowed to take place for 72 hr at 25°. Stirring was achieved by a magnetic stirrer and by slow aeration. The discs were filtered off and extracted with MeOH (150 ml); isolation of the extract and separation in the usual way gave a neutral fraction (424 mg) and a phenolic fraction (223 mg). TLC separation of the phenolic fraction at R_f = 0·3 (silica gel, benzene–EtOAc 3:1) gave gentisein (11 mg), at R_f = 0·58–0·60 gentisin and iso-gentisin (46 mg), and at R_f = 0·1 an oil (3 mg) corresponding to where benzophenone would be located.

The mixtures of xanthones (57 mg) was demethylated and the residue purified by TLC to give gentisein (23 mg). This xanthone was repurified to give a constant activity of 20,650 dpm/mg, i.e. a specific activity of 506 dpm/μ mole. The radio-active incorporation based on the xanthones isolated was 0·53%.

Dilution of the fraction corresponding to benzophenone with inactive 2,3′,4,6-tetrahydroxybenzophenone and recrystallized from MeOH–benzene gave the benzophenone with a constant activity of 449 dpm/mg. These activities give a value for the concentration of radio-active naturally occurring II (R = R′ = R″ = H) as 1·20 mg, i.e. a 0·0005% content in the rhizome.

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REFERENCES

[1] Henry and Caventou, *J. Pharm. Chim.* **7**, 178 (1821) quoted by reference 2*b*.
[2] [a] H. Kostenecki and J. Tambor, *Monatsh.* **15**, 1 (1894);
[b] *Ber. Dtsch. Chem. Ges.* **27**, 190 (1894).
[3] A. G. Perkin, *J. Chem. Soc.* **73**, 1028 (1898).
[4] N. Anand and K. Venkataraman, *Proc. Ind. Acad. Sci.* 438 (1947).
[5] L. Cannonica and F. Pelizzoni, *Gazzetta* **85**, 1007 (1955).
[6] P. K. Grover, G. D. Shah and R. C. Shah, *J. Chem. Soc.* 3982 (1955).
[7] J. R. Lewis, *Proc. Chem. Soc.* 373 (1963).
[8] J. R. Lewis and B. H. Warrington, *J. Chem. Soc.* 5074 (1964).
[9] J. E. Atkinson and J. R. Lewis, *Chem. Comm.* 803 (1967).
[10] R. C. Ellis, W. B. Whalley and (in part) K. Ball, *Ibid.* 803 (1967).
[11] J. E. Atkinson and J. R. Lewis, *J. Chem. Soc.* forthcoming publication.
[12] H. D. Locksley, I. Moore and F. Scheinmann, *Tetrahedron* **23**, 2234 (1967).