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STUDIES ON THE CHEMICAL COMPONENTS OF THE RUTACEAE PLANTS—VII[1]

COMPONENTS OF THE ROOTS OF *PONCIRUS TRIFOLIATA* RAFINESQUE (5). PONCITRIN, A NEW COUMARIN: STRUCTURE AND CHEMICAL DEGRADATION

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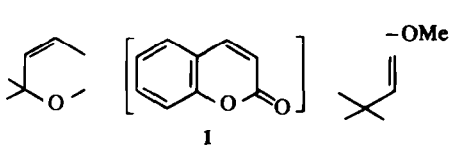
Abstract—The structure of poncitrin, a new coumarin, isolated from the roots of *Poncirus trifoliata* RAFINESQUE, has been established as **2**, by chemical degradation.

In previous papers,[1,2] we described the isolation of poncitrin from the roots of *Poncirus trifoliata* RAFINESQUE, and proposed formula **1** as a partial structure for this new natural coumarin on the basis of spectroscopic studies. Furthermore, of twelve possible structures,[2] formula **2** was determined for poncitrin by the use of intramolecular inter-nuclear Overhauser effects. This paper presents a further study of the structure of poncitrin (**2**) by chemical degradation.

For establishing the configuration of the O atoms in **2**, alkali-fusion of **2** with KOH was carried out to furnish a phloroglucinol derivative, methylation of which with diazomethane gave a product identified by GLC as phloroglucinol trimethyl ether. The present data suggested three possible structural formulae, **2**, **3**, and **4** for poncitrin.

Oxidation of **2** with $KMnO_4$ in an alkaline solution produced α-hydroxyisobutryic acid. The product was identified with an authentic sample by mixed m.p. determination and comparison of the gas–liquid chromatograms of the Me_4Si derivatives of their methyl and ethyl esters. This reaction[3] supports the assumption that poncitrin contains the 2, 2-dimethylchromene ring as indicated by its ^1H NMR spectrum.[1,2] In addition, the fact that formaldehyde was produced from **2** by a modified Lemieux–Rudloff test[4] indicates the presence of a



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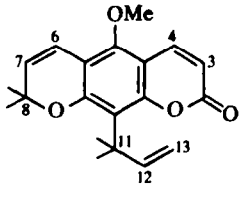

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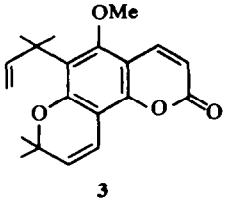

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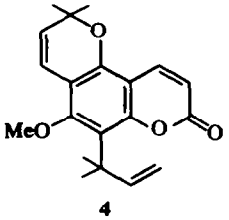

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CHART 1

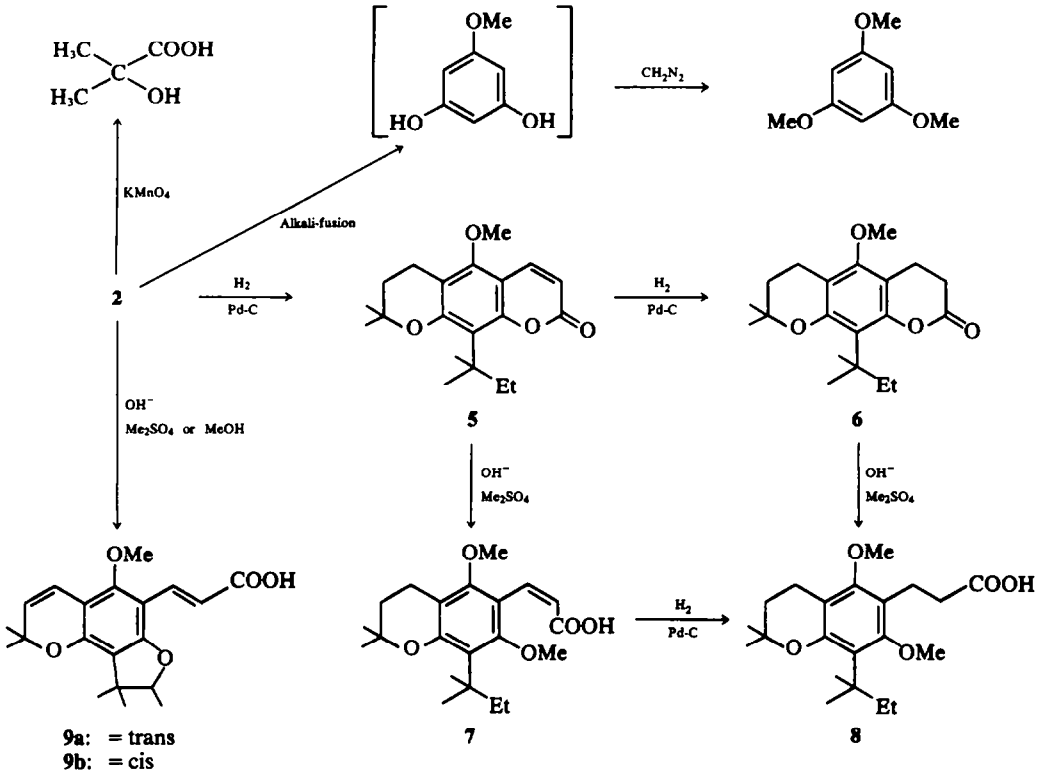

CHART 2

non-conjugated double bond in the terminal position of **2**.

It has already been reported that poncitrin has two reducible double bonds.[1,2] However, hydrogenation of poncitrin over Pd-catalyst in glacial AcOH under certain strict conditions gave hexahydroponcitrin (**6**). The IR and UV spectra show the absence of the coumarin ring. Its mass spectrum has a parent peak at *m/e* 332. The NMR spectrum shows signals corresponding to two equivalent *gem*-Me_2 at δ 1·32 and 1·50, one OMe at δ 3·70, one Et at δ 0·68 (t) and 1·87 (q), and two A_2B_2-type —CH_2CH_2— groups at δ 1·72 and 2·70 (t), and around 2·73 (m). These data indicate a compound with structure **6**, hydrogenation of **2** having proceeded stepwise to give tetrahydroponcitrin (**6**)[1,2] and then the hexahydro derivative.

The standard procedure for converting a coumarin into the O-methyl ether of its corresponding cinnamic acid was carried out on **5**, which was converted into O-methyltetrahydroponcitrinic acid (**7**). Its mass spectrum has a parent peak at *m/e* 362. Its NMR spectrum shows the presence of an additional OMe (δ 3·50) and an OH group (δ 8·90). Other spectral features are similar to those for **5**.[1,2] A J-value of 12·3 Hz obtained from an AB-type quartet at δ 6·02 and 6·98 due to olefinic protons implies that **7** has a *cis*-substituted double bond. The *trans* double bond has a J-value of 16·0 Hz as described later.

Compound **7** was readily hydrogenated with Pd-catalyst to form O-methylhexahydroponcitrinic acid (**8**). In its NMR spectrum, the AB-type quartet seen in the spectrum of **7** has disappeared. Treatment of **6** with 20% aqueous NaOH and Me_2SO_4 also gave **8**, shown to be identical with the product prepared from **5** through **7** by mixed m.p. determination and a comparison of their IR and NMR spectra. It was thus confirmed that **5** has a coumarin nucleus.

Treatment of **2** with 50% aqueous KOH afforded compound A, m.p. 215–216°. On the other hand, treatment of **2** with 20% aqueous NaOH in MeOH afforded compound B, m.p. 154–155°. In this reaction, the influence of sunlight had to kept away, otherwise polymerisation was caused. Moreover, another isomeric derivative, compound C, m.p. 153–155°, was obtained from **2** by the same procedure as used for the conversion of **5** and **6** into **7** and **8**, respectively. The molecular formula, $C_{20}H_{24}O_5$, was found to be the same for all of these three compounds by their elemental analyses and by molecular weight calculations from their mass spectra. Then, the correlation of these three compounds was disclosed by the following observation.

The NMR spectrum of compound A shows

five Me signals at δ 1·17 (s), 1·40 (s), 1·42 (6H, s), and 1·40 (d); an OMe signal at δ 3·75; a methine quartet at δ4·47; two AB-type quartets due to olefinic protons at δ 5·52 and 6·55 (J=9·8 Hz), and 6·78 and 7·97(J=16·0 Hz); and a broad band due to COOH at δ 8·44. These results suggested the presence of a new ring in the cinnamic acid derivative. It is reasonable to conclude that on the conversion of the coumarin into the O-hydroxycinnamic acid derivative, an intramolecular transformation[5] between the phenolic group and the terminal double bond occurred to form a new ring. On the other hand, the NMR spectrum of compound B shows five Me signals at δ 1·12 (s), 1·35 (s), 1·28 (d), and 1·40 (6H, s); one OMe signal at δ 3·70; a methine quartet at δ 4·30; two AB-type quartets at δ 5·43 and 6·47 (J = 9·8 Hz), and at 5·93 and 6·87 (J = 12·0 Hz); and one broad OH signal at δ 10·30. Thus, the signals in both spectra were revealed to be very similar to each other except for J-values between the olefinic protons; in the former the J-value is 16·0 Hz, whereas it is 12·0 Hz in the latter. Furthermore, in the UV spectrum of compound B, the maximum absorption is shifted to a shorter wave length by 14 nm in comparison with that at 282 nm in the spectrum of compound A. These data indicate that compounds A and B can be assigned to *trans*- and *cis*-cycloponcitrinic acids (**9a** and **9b**), respectively.

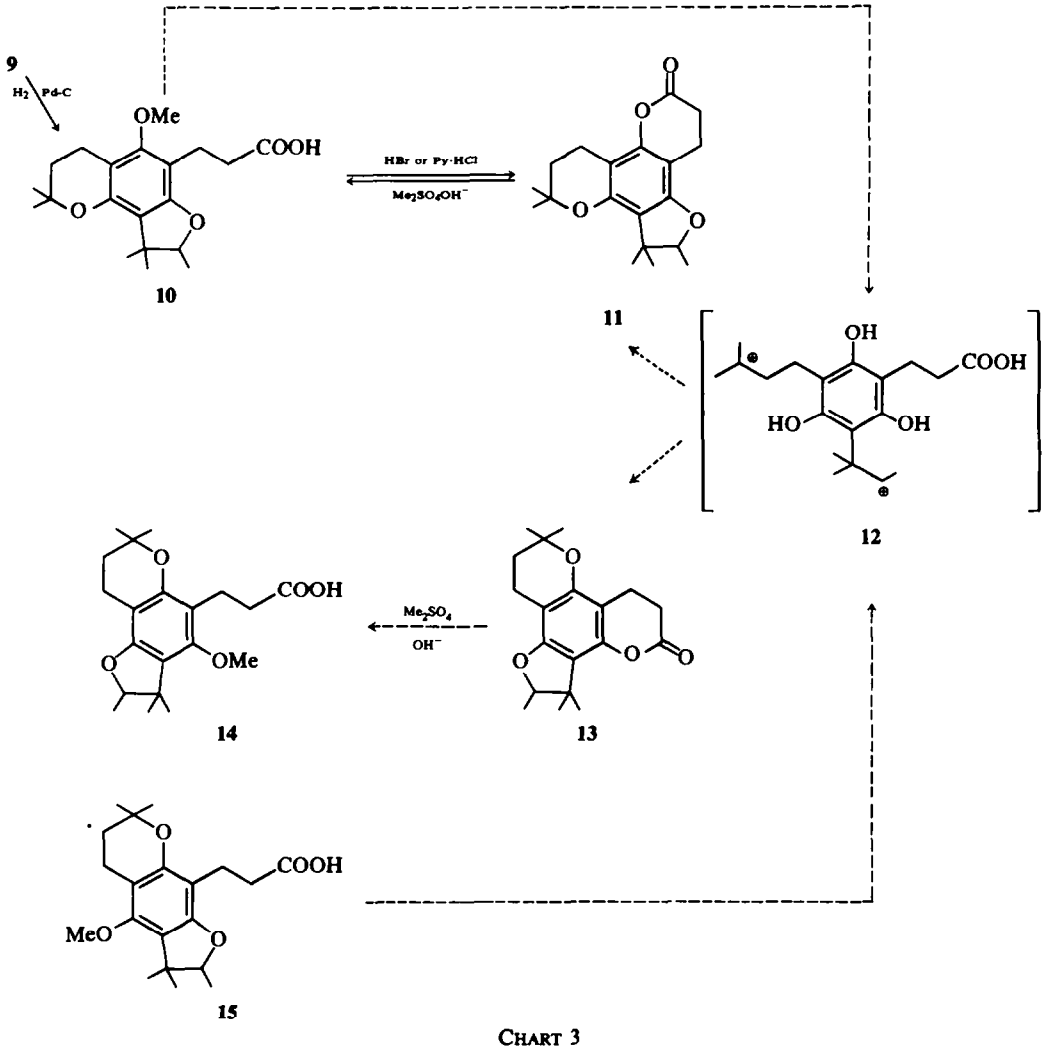

CHART 3

In the NMR spectrum of compound C, there appear four singlets at δ 1·12, 1·17, 1·36, and 1·40 and two doublets at δ 1·30 and 1·40 arising from six Me; a singlet at δ 1·40 due to four Me; two singlets at δ 3·70 and 3·75 due to two OMe; two methine quartets at δ 4·32 and 4·44; four AB-type quartets at δ 5·46 and 6·51 (J = 9·8 Hz), 5·52 and 6·54 (J = 9·8 Hz), 5·96 and 6·90 (J = 12·0 Hz), and 6·76 and 7·95 (J = 16·0 Hz); and an OH signal at δ 10·13.

Apparently these signals consist of those shown in the spectra of compounds A and B. Consequently, compound C was elucidated to be a 1:1 molecular complex of *trans*- and *cis*-cycloponcitrinic acids (**9a** and **9b**, respectively). This finding was also supported by the fact that compound B was changed into compound C by treatment with 20% aqueous NaOH.

Hydrogenation of each of compounds A, B, and C with Pd-catalyst afforded samples of terahydro derivative (**10**), which were identified with each other by comparison of their IR, NMR, and mass spectra and by mixed m.p. determination. The NMR spectrum of **10** shows no olefinic proton signals; instead, two A_2B_2-type $—CH_2CH_2—$ signals appear as two triplets at δ 1·75 and 2·67, and a multiplet at around δ 2·73.

From the above results, formula **3** has been ruled out because poncitrin was made to form the cycloponcitrinic acids. Therefore, tetrahydrocycloponcitrinic acid can be depicted by two tentative structures, **10** and **15**. The preference of formula **10** will be shown as follows.

Demethylation of **10** with 47% HBr under strictly controlled conditions gave rise to a compound with m.p. 117–119° and *m/e* 316 (M^+) in a comparatively good yield. The product showed one spot on TLC, and one peak on GLC. The IR absorption band at 1760 cm^{-1} indicated the presence of a $\gamma\delta$ unsaturated δ-lactone. The NMR spectrum in $CDCl_3$ showed signals of appropriate intensities corresponding to five Me [δ 1·12 (s), 1·39 (s), 1·32 (6H s), and 1·34 (d)], two A_2B_2-type $—CH_2CH_2—$ groups (δ 1·76 and 2·68 as triplets, and around 2·77 as multiplets), and one methine proton (δ 4·31, q). The NMR spectrum in C_6D_6 was also examined, with easier signal-assignments. Benzene-induced solvent shifts[6] for the $—CH_2CH_2—$ triplets at δ 1·76 and 2·68 in $CDCl_3$ were −0·34 and −0·08, respectively; this fact implies the formation of a new lactone ring, proximate to which the C-6 methylene protons giving the latter signal must be situated. These findings suggested that the structure of the lactone is represented by formula **11**.

However, here arises the question whether the lactone was derived via an intermediate **12** from **10** or not. If it was so derived, the product lactone may be a mixture of compounds **11** and **12**, and this mixture should also be derivable from **15** as well as from **10**.

In order to elucidate this question, the following reactions were carried out. Treatment of the lactone **11** with 20% aqueous NaOH and Me_2SO_4 followed by hydrolysis by the usual procedure really afforded the starting monobasic acid **10** in an almost quantitative yield. It should be emphasised that the different acid **14** was not obtained at all. As a result, the structure of the lactone must be designated as **11**. Further, treatment of **10** with pyridine hydrochloride[7] produced the same lactone **11**. The identity of the product was confirmed by a mixed m.p. determination and a comparison of their IR and NMR spectra.

Finally, the following observations on the former demethylation monitored by IR spectroscopy may provide conclusive evidence that the lactone **11** was not derived via **12**. Acid **10** has a characteristic IR band at 1704 cm^{-1} corresponding to the CO group, whereas lactone **11** has the band at 1760 cm^{-1}, as described above. A mixture of **10** and **11** in a 1:1 weight ratio clearly shows both IR bands at 1704 and 1760 cm^{-1}. The C—O—C absorption bands of these compounds appear in the 1200–1100 cm^{-1} region. Changes in the IR spectra on refluxing **10** with 47% HBr were followed by taking measurements at 20 min intervals. A linear relationship between the logarithm of the optical density ratio of the 1704 cm^{-1} band and the 1602 cm^{-1} aromatic C=C internal-standard band, D_{1704}/D_{1602} of **10**, and reaction time was first order within 80 min. On the other hand, the ratios of D_{1140}/D_{1602} and D_{1124}/D_{1602} for the bands due to the 5- and 6-membered ethers were almost constant, indicating that the ether links were not changed within 80 min. After 100 min refluxing, however, a decrease in the intensity of the 1760 cm^{-1} band was observed, suggesting that ring-opening of the lactone occurred. The relative optical densities of the cyclic ether bands simultaneously decreased by degrees. It is therefore apparent that **10** is converted into lactone **11** during the first 80 min, and that the lactone is then converted into another or other compounds by further heating. Thus, the assumption that the demethylation proceeded via an intermediate such as **12** can be ruled out under the present experimental conditions.

In conclusion, chemical degradation has shown that poncitrin should be assigned the structure **2** a result which is in agreement with that deduced from the study of nuclear Overhauser effects.[1,2]

EXPERIMENTAL

M.ps were determined on a Yanaco Micro Melting Point Apparatus and are uncorrected. Microanalyses were performed at the Elemental Analysis Centre of this Faculty (University of Tokushima). UV spectra were obtained with a Hitachi Model 124 spectrophotometer. IR spectra were taken with a Hitachi EPI-G-2 spectrophotometer. NMR spectra were recorded on a Varian A-60A spectrometer, using *ca* 5% (w/v) solutions of compounds in $CDCl_3$ with Me_4Si as an internal standard, except where stated otherwise. Mass spectra were measured with a Hitachi Model RMV-6E spectrometer.

Alkali-fusion of poncitrin (**2**). A mixture of **2** (1·416 g) and KOH (3·64 g) in a nickel crucible was heated on an oil bath at 300° for 1 h; then cooled, acidified with HCl aq, and separated into 3 fractions: neutral, phenolic, and carboxylic acid, by the usual procedure. The phenolic fraction was methylated using a soln of diazomethane in Et_2O. The methyl ether was compared and identified with an authentic sample of phloroglucinol trimethyl ether by

GLC; sample 0·84 min (3% SE-30),* 31·18 min (30% DDP);† standard 0·84 min (3% SE-30), 31·16 min (30% DDP).

α-Hydroxyisobutyric acid. To a soln of **2** (480 mg) in 25 ml MeOH 7 ml of 3% aq NaOH was added. The MeOH was removed under reduced pressure and 300 ml of 1% $KMnO_4$ aq was added to the remaining alkaline soln with very vigorous agitation over a period of 30 min. The mixture was warmed for 30 min on a steam bath, decolourised with SO_2, and then extracted with Et_2O. The ether extract was treated in the usual manner to give a residue as colourless needles, m.p. 75–77°. There was no depression of m.p. upon admixture with an authentic sample of α-hydroxyisobutyric acid. For purposes of GLC comparison† of this compound with authentic material, a sample was methylated and another sample ethylated using solutions of diazomethane and diazoethane, respectively, in Et_2O. The methylated and ethylated samples were then allowed to react with a soln of Me_4Si in pyridine, to form the methyl ester-Me_4Si and the ethyl ester-Me_4Si derivatives. The following retention times, relative to pyridine, were obtained: methyl ester-Me_4Si derivative; sample 0·19 min, standard 0·19 min; ethyl ester-Me_4Si derivative; sample 0·22 min, standard 0·22 min.

The modified Lemieux–Rudloff test. To a soln of 6 mg of **2** dissolved in a mixture of 17 ml distilled water and 2 ml pyridine was added 10 ml 0·02M $NaIO_4$ and 1 ml 0·005M $KMnO_4$. The solns were allowed to react for 30 min at room temp. To 1 ml of this soln was added 1 ml of a soln containing 200 mg of chromotropic acid in 100 ml 6N H_2SO_4 and the mixture was heated for 1 h on a steam bath. A positive result in this test is the formation of a violet colour, indicating the production of formaldehyde. A comparative test by this colour reaction gave the following results: cinchonine, positive; styrene, positive; tetrahydroponcitrin, negative.

Hexahydroponcitrin (**6**). Poncitrin **2** (500 mg) was mixed with 30 ml glacial AcOH and 300 mg of 10% Pd-C catalyst. Hydrogenation was carried out at room temp and atmospheric pressure until no more H_2 was consumed. The catalyst was removed by filtration, and the AcOH was removed by distillation under reduced pressure leaving a residue. Recrystallisation of the residue from EtOH gave 495 mg (99%) of colourless needles, m.p. 97–98°; λ_{max}^{EtOH}: 292 nm; ν_{max}^{KBr}: 1759 (C=O), 1590 (C=C) cm^{-1}; NMR, see text. (Found: C, 72·65; H, 8·78. Calcd for $C_{20}H_{28}O_4$: C, 72·26; H, 8·49%).

O-Methyltetrahydroponcitrinic acid (**7**). To a warm solution of **5** (197 mg) in 5 ml MeOH was added 30 ml of 20% NaOH aq and the mixture was warmed for 5 min on a steam bath. An additional 15 ml of 20% NaOH aq was added to the mixture, and Me_2SO_4 was gradually added with very vigorous agitation until the mixture became neutral. Alternate addition of alkali and Me_2SO_4 was repeated 3 times. Finally, the mixture was shaken vigorously for 1 h at room temp, the MeOH was removed by distillation under reduced pressure, and the remaining soln was extracted exhaustively with Et_2O. The ethereal extract was dried over Na_2SO_4, and the ether was removed on a steam bath. To the residue, 20 ml KOH 70% EtOH containing 1·2 g was added, and the mixture was heated for 1 h on a steam bath. The mixture was then allowed to cool and conc HCl was added until the mixture became acid. The acid soln was exhaustively extracted with Et_2O. The ether extract was extracted with 5% Na_2CO_3 aq. The Na_2CO_3 extract was acidified with conc HCl and exhaustively extracted with Et_2O. The ether extract was dried over Na_2SO_4, and the ether was removed on a steam bath. A crystalline residue remained, which was recrystallised from MeOH to give 164 mg (83%) of colourless needles, m.p. 168–169°; ν_{max}^{KBr}: 1683 (C=O), 1630 (C=C), 1580 (aromatic C=C) cm^{-1}; NMR δ: 0·65 (3H, s, Me), 1·30 (6H, s, gem-Me_2), 1·42 (6H, s, gem-Me_2), 1·70 (2H, t, CH_2), 1·83 (2H, q, CH_2), 2·70 (2H, t, CH_2), 3·50 (3H, s, OMe), 3·60 (3H, s, OMe), 6·02 and 6·98 (2H, AB type, CH=CH), 8·90 (1H, b, OH). (Found: C, 69·42; H, 8·30. Calcd for $C_{21}H_{30}O_5$: C, 69·58; H, 8·34%).

O-Methylhexahydroponcitrinic acid (**8**). **A.** A soln of **7** (145 mg) in AcOH was hydrogenated according to the method used in the preparation of **6**. The mixture was filtered and evaporated to dryness, and the residue was recrystallised from a mixture of EtOH and light petroleum to give 140 mg (96%) colourless needles, m.p. 126–127°; λ_{max}^{EtOH}: 281, 288 nm; ν_{max}^{KBr}: 1700 (C=O), 1580 (aromatic C=C) cm^{-1}; NMR δ: 0·67 (3H, t, Me), 1·31 (6H, s, gem-Me_2), 1·45 (6H, s, gem-Me_2), 1·70 (2H, t, CH_2), ~2·7 (4H, A_2B_2, CH_2CH_2), 1·88 (2H, q, CH_2), 2·80 (2H, t, CH_2), 3·60 (3H, s, OMe), 3·72 (3H, s, OMe), 9·87 (1H, b, OH). (Found: C, 69·48; H, 8·84. Calcd for $C_{21}H_{32}O_5$: C, 69·20; H, 8·85%). **B.** A soln of **6** (100 mg) in MeOH was allowed to react with 20% NaOH aq and Me_2SO_4 according to the method used in the preparation of **7**. Recrystallisation of the residue from a mixture of EtOH and light petroleum gave 85 mg (85%) colourless needles, m.p. 126–127°. The IR and NMR spectra were superimposable on those of a sample of **8** prepared from **7** by method A. There was no depression of m.p. upon admixture with a sample of **8**. (Found: C, 68·89; H, 8·75. Calcd for $C_{21}H_{32}O_5$: C, 69·20; H, 8·85%).

trans-*Cycloponcitrinic acid* (**9a**). **A.** A mixture of **2** (305 mg) and 10 ml of a 50% KOH aq was heated on an oil bath at 140–170° for 10 min, 170–200° for 20 min, and 210–275° for 10 min, then cooled, acidified with HCl aq (Congo red), and extracted exhaustively with Et_2O. Following treatment with 5% $NaHCO_3$ aq, the ethereal extract was dried over Na_2SO_4. Evaporation of the solvent gave a crystalline residue, which upon recrystallisation from MeOH or EtOH yielded 280 mg colourless prisms, m.p. 215–216°; λ_{max}^{EtOH}: 282 nm; NMR, see text. (Found: C, 69·86; H, 7·20. Calcd for $C_{20}H_{24}O_5$: C, 69·75; H, 7·02%). **B.** A soln of **2** (385 mg) in MeOH was allowed to react with 20% NaOH aq and Me_2SO_4 as in method A, except that the mixture was agitated vigorously for 1 h at 35°. Recrystallisation of the residue from MeOH gave 300 mg (77%) colourless meedles, m.p. 215–217°; ν_{max}^{KBr}: 1670 (C=O), 1608 (C=C), 1590 (aromatic C=C); The IR, NMR, and mass spectra were all in complete agreement with those of an authentic sample of **9a**. (Found: C, 69·62; H, 6·94.

*Apparatus; Hitachi F6D Gas-chromatograph. Column; stainless steel, 1 m × 3 mm i.d., containing 3% SE-30 on WAW (30–60 mesh). Instrumental parameters; isothermal operation at a column temperature of 160°, an injection temperature of 190°, and a detector temperature of 190°. Carrier gas; N_2 with a flow rate of 0·75 Kg/min. Chart speed; 10 mm/min.

†Apparatus; Hitachi F6D Gas-chromatograph. Column; stainless steel, 1 m × 3 mm i.d., containing 30% di-n-decylphthalate coated on celite 545 (60–80 mesh). Instrumental parameters; isothermal operation at a column temperature of 120°, an injection temperature of 190°, and a detector temperature of 190°. Carrier gas; N_2 with a flow rate of 0·9 Kg/cm^2. Chart speed; 5 mm/min.

Calcd for $C_{20}H_{24}O_5$: C, 69·75; H, 7·02%). By this method, **9a** was produced exclusively.

cis-Cycloponcitrinic acid (**9b**). To a warm soln of **2** (339 mg) in 50 ml MeOH 20 ml 20% NaOH aq was added and the mixture warmed for 5 min on a steam bath. After the mxiture had been stirred for 1 h at room temp, the MeOH was removed by distillation under reduced pressure. After acidification with HCl aq (Congo red), extraction of the aqueous liquor gave 348 mg (97%) colourless prisms, m.p. 154–155°; λ_{max}^{EtOH}: 237, 268 nm; NMR see text. (Found: C, 69·96; H, 7·22. Calcd for $C_{20}H_{24}O_5$: C, 69·75; H, 7·02%). By this method, **9b** was prepared exclusively.

trans- *and* cis-*Cycloponcitrinic acids* (**9a** *and* **9b**). **A.** A soln of **2** (300 mg) in MeOH was allowed to react with 20% NaOH aq and Me_2SO_4 according to the method used in the preparation of **7** and **8**. Recrystalisation of the residue from MeOH gave 290 mg of colourless prisms, m.p. 153–155°; ν_{max}^{KBr}: 1690 (C=O), 1605 (C=C), 1590 (aromatic C=C); NMR, see the text. (Found: C, 69·62; H, 7·23. Calcd for $C_{20}H_{24}O_5$: C, 69·75; H, 7·02%). A 1:1 molecular complex of *trans*- and *cis*-cycloponcitrinic acids (**9a** and **9b**, respectively) was produced. **B.** A soln of **9b** (10 mg) in 15 ml 20% NaOH aq was heated at boiling on an oil bath for 1 h. After acidification with HCl aq (Congo red), extraction of the aqueous liquor gave 7 mg of colourless prisms, m.p. 153–155°. λ_{max}^{EtOH}: 274 nm. The NMR was superimposable on that of the sample obtained by method A. There was no depression of melting point upon admixture with the sample.

Cyclotetrahydroponcitrinic acid (**10**). **A.** A soln of 175 mg of **9a** in AcOH was hydrogenated according to the method used in the preparation of **6**. The mixture was filtered and evaporated to dryness, and the residue was recrystallised from a mixture of EtOH and light petroleum to give 160 mg colourless needles, m.p. 145–146°; λ_{max}^{KBr}: 1700 (C=O), 1600 (aromatic C=C) cm^{-1}; NMR δ: 1·10 (3H, s, Me), 1·30 (3H, d, Me), 1·32 (6H, s, gem-Me_2), 1·37 (3H, s, Me), 1·75 (2H, t, CH_2), 2·67 (2H, t, CH_2), ~2·73 (4H, A_2B_2, CH_2CH_2), 3·71 (3H, s, OMe), 4·30 (2H, q, CH_2). (Found: C, 68·57; H, 8·14. Calcd for $C_{20}H_{28}O_5$: C, 68·94; H, 8·10%). **B.** A soln of 330 mg of the *cis*-acid **9b** prepared above was dissolved in AcOH, and then hydrogenated in an almost quantitative yield as in method A. The identity of this hydrogenated product with **10** was confirmed by a mixed m.p. determination and comparison of the IR and NMR spectra of the sample obtained by method A. (Found: C, 68·85; H, 8·50. Calcd for $C_{20}H_{28}O_5$: C, 68·94; H, 8·10%). **C.** A soln of 100 mg of a 1:1 molecular complex of **9a** and **9b** in AcOH was hydrogenated as in method A. Recrystallisation of the residue gave 95 mg (95%) of colourless needles, m.p. 145–146°. The IR and NMR spectra of the sample of **10** prepared here were superimposable on those of the samples of **10** prepared from **9a** and **9b** by methods A and B. There was no depression of m.p. upon admixture with the samples of **10** prepared by methods A and B. (Found: C, 68·75; H, 8·21. Calcd for $C_{20}H_{28}O_5$: C, 68·94; H, 8·10%).

Lactone **11**. **10** (200 mg) was added to a soln of 83 mg of red P in 17 ml of 47% HBr. The mixture was heated at boiling for 1·5 h, cooled by pouring onto ice, and then extracted exhaustively with Et_2O. Following treatment with 5% $NaHCO_3$ aq, the ethereal extract was dried over $MgSO_4$ and the solvent was removed by evaporation. The residue was recrystallised from MeOH to yield 148 mg (82%) colourless needles, m.p. 117–119°. Reactions under other conditions were as follows: **10** (240 mg), HBr (20 ml), red P (100 mg), with a reaction time of 120 min yielded 154 mg (70·6%) of **11**; **10** (277 mg), HBr (23 ml), red P (123 mg), with a reaction time of 150 min yielded 156 mg (56·4%) of **11**. The TLC exhibited one spot with R_f 0·85 on an Al_2O_3 plate (in toluene:ethyl formate:formic acid = 5:4:1; by use of iodine). The GLC exhibited one peak with retention time 10·43 min (1·5% SE-30);* λ_{max}^{EtOH}: 287 nm; IR and NMR, see test. (Found: C, 72·20; H, 7·79. Calcd for $C_{19}H_{24}O_4$: C, 72·12; H, 7·65%).

Ring-opening of lactone **11**. To a warm soln of **11** (228 mg) in 30 ml MeOH was added 15 ml 20% NaOH aq, and the mixture was warmed on a steam bath for 5 min. After 25 ml of NaOH aq had been added to the reaction, Me_2SO_4 was gradually introduced with very vigorous agitation until the mixture became neutral. The mixture was then kept at room temp for 1 h, and extracted with Et_2O. The solid left after removal of the solvent was boiled with 18 ml 70% EtOH containing KOH (2 g) for 1 h. After acidification with HCl aq (Congo red), the EtOH was removed by distillation under reduced pressure, and the residue extracted with Et_2O. Following treatment with 5% Na_2CO_3 aq, the ether extract was dried over $MgSO_4$. Evaporation of the ether left a crystalline residue, which upon recrystallisation from MeOH yielded 240 mg (95·6%) colourless needles, m.p. 145–146°. The IR and NMR spectra were superimposable on those of a sample of **10** prepared as above. There was no depression of m.p. upon admixture with the authentic sample. (Found: C, 68·76; H, 8·23. Calcd for $C_{20}H_{28}O_5$: C, 68·94; H, 8·10%).

Cleavage of the methyl ether group in **10** *by pyridine hydrochloride*. **10** (82 mg) was added to a soln of pyridine hydrochloride (340 mg) in 20 ml decalin, and the mixture was heated at boiling for 5 h. The mixture was then poured into cold water and the decalin layer separated from the aqueous soln, which was then acidified with HCl aq, and extracted with $CHCl_3$. The decalin was removed by distillation under reduced pressure, and the residue obtained was dissolved in $CHCl_3$. The combined $CHCl_3$ soln was extracted with 5% Na_2CO_3 aq, and dried over $MgSO_4$. Removal of $CHCl_3$ by distillation afforded 39 mg of **11**. Crystallisation from MeOH gave a compound with m.p. 117–119°, undepressed on admixture with an authentic sample of **11**. Its IR and NMR spectra were identical with those of an authentic sample. The starting material **10** was recovered from the Na_2CO_3 aq by the usual procedure. Recrystallisation from MeOH gave 30 mg of **10**; m.p. 143–145°, undepressed on admixture with an authentic sample of **10**. The respective IR and NMR spectra were identical.

Demethylation of **10** *monitored by* IR *spectroscopy*. **10** (157 mg) was added to a soln of red P (60 mg) in 13 ml 47% HBr, and the mixture was heated at boiling on an oil bath for 160 min. Small amounts of the mixture were taken out at 20 min intervals, cooled by pouring onto ice, extracted with $CHCl_3$, and dried over $MgSO_4$. The solvent was then removed by evaporation. The IR spectrum of each residue was measured in $CHCl_3$.

*Apparatus; Hitachi F6D Gas-chromatograph. Column; stainless steel, 1 m × 3 mm i.d., containing 1·5% SE-30 on chromosorb WAW (60–80 mesh) was used. Instrumental parameters; isothermal operation at a column temp of 170°, an injection temp of 220°, and a detector temp of 220°. Carrier gas; N_2 with a flow rate of 1·27 Kg/cm². Chart speed; 5 mm/min.

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