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

COMPONENTS OF THE ROOT OF *PONCIRUS TRIFOLIATA* RAFINESQUE (4) PONCITRIN, A NEW COUMARIN: STRUCTURE AND NUCLEAR OVERHAUSER EFFECTS[2]

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Abstract—A new coumarin, $C_{20}H_{22}O_4$, named poncitrin, was isolated from the root of *Poncirus trifoliata* RAFINESQUE. The structures of poncitrin (III) and tetrahydroponcitrin (XV) have been elucidated, mainly by means of NMR spectroscopy including intramolecular nuclear Overhauser effect measurements.

DURING A CURRENT project dealing with the constituents of *Poncirus trifoliata* RAFINESQUE,[3] a fluorescent crystalline substance was obtained from the methanol extract of the root. It was identified as a new coumarin and named poncitrin. The present paper describes the structure of poncitrin as determined mainly by NMR spectroscopy including the intramolecular nuclear Overhauser effect (NOE).[4]

The molecular formula $C_{20}H_{22}O_4$ of poncitrin (colourless pillars, m.p. 93–94°, optically inactive) was established by elementary analysis together with a molecular weight determination by mass spectrometry. The presence of an OMe group was revealed by the Vieböck–Brecher method. The UV spectrum shows four peaks at 266, 274, 331, and 351 nm, as shown in Fig 1a, and its IR spectrum has two high-intensity carbonyl bands at 1720 and 1608 cm^{-1} due to an $\alpha\beta$-unsaturated δ-lactone. These findings suggest that poncitrin is a coumarin having an OMe group.

Hydrogenation of poncitrin in glacial AcOH over Pd-C catalyst gave tetrahydroponcitrin (colourless prisms, m.p. 94–95°) which has the molecular formula $C_{20}H_{26}O_4$ determined by elementary and mass spectral analyses. The UV spectrum is significantly different from that of poncitrin, as shown in Fig. 1b, while its IR spectrum still has the bands characteristic of the coumarin nucleus.[5] Accordingly, one of the double bonds in poncitrin was concluded to be conjugated with the main chromophore.

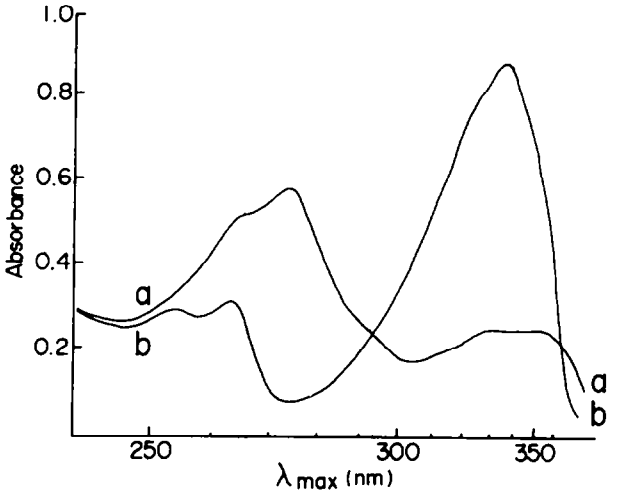


FIG 1. UV spectra of (a) poncitrin (III) and (b) tetrahydroponcitrin (XV) in EtOH.

The 100-MHz NMR spectrum of poncitrin in $CDCl_3$ is shown in Fig 2a. Doublets of an AX-type at δ 7·86 and 6·16 ppm (J = 9·8 Hz) arise from the C-4 and C-3 protons in the coumarin nucleus, respectively. A sharp singlet at δ 3·82 ppm is attributed to the OMe group. Another sharp singlet at δ 1·46 ppm and an AB-type quartet (δ 6·57 and 5·69 ppm, J = 10·2 Hz) can be assigned to the *gem*-Me_2 and

TABLE 1. NUCLEAR OVERHAUSER EFFECTS (%)[a,b] AND CHEMICAL SHIFTS (δ, PPM DOWNFIELD FROM TMS)[c] OBSERVED FOR PONCITRIN (III) IN $CDCl_3$[d]

Saturated signals		Observed signals						
		3-H	4-H	6-H	7-H	12-H	13-H(cis)	13-H(trans)
	δ	6·16	7·86	6·57	5·69	6·30	4·93	4·87
		(5·92)	(7·35)	(6·47)	(5·32)	(6·43)	(5·03)	(4·93)
5-OMe		−7	14	14	−4	0	0	0
δ 3·82								
(3·30)		(−6)	(14)	(15)	(−5)	(0)	(0)	(0)
8-Me_2		0	0	−8	22	7	0	0
δ 1·46								
(1·22)		(0)	(0)	(*d*)	(22)	(8)	(0)	(0)
11-Me_2		0	0	0	0	9	13	−7
δ 1·67								
(1·80)		(0)	(0)	(0)	(0)	(*d*)	(*d*)	(−5)
		20[4-H][e]	18[3-H][e]	13[7-H][e]	16[6-H][e]			

[a] The NOE data preliminarily reported[2] are completely revised in the present study.

[b] Increases in signal heights are shown in parentheses.

[c] Figures in parentheses are those obtained in C_6D_6.

[d] The double resonance experiments revealed the long-range spin couplings, $J_{7,8\text{-Me}}$, $J_{12,11\text{-Me}}$, and $J_{13(cis),11\text{-Me}}$. Therefore, in these cases, increases in signal heights are not shown.

[e] In these cases, saturated signals are shown in square brackets.

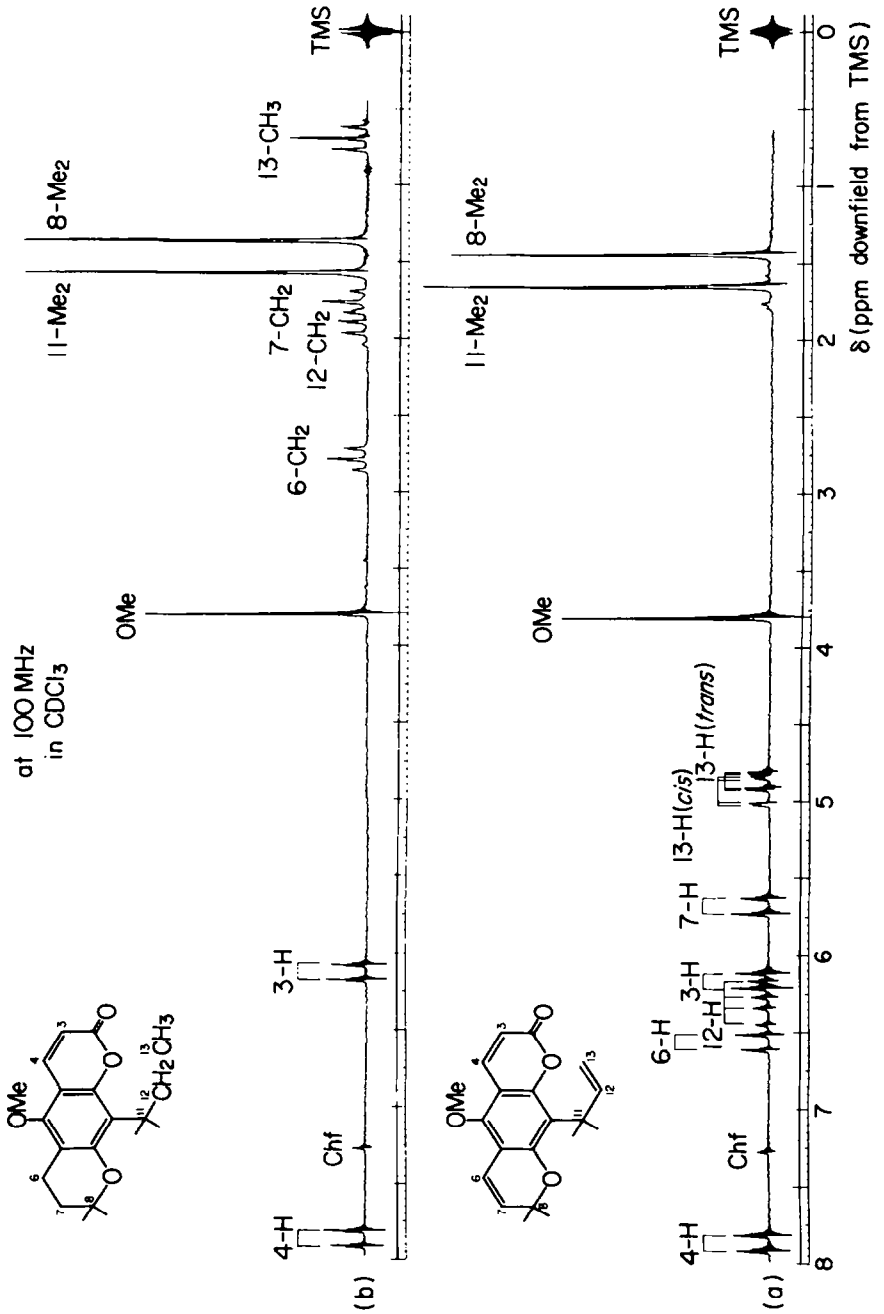


FIG 2. NMR spectra of (a) poncitrin (III) and (b) tetrahydroponcitrin (XV) in $CDCl_3$ at 100 MHz.

to 4-H and 3-H of a 2,2-dimethylchromene ring, respectively. Therefore, the presence of a coumarin nucleus, an OMe group, and a 2,2-dimethylchromene ring leaves a C_5H_9 fragment unaccounted for.

Thus, a singlet signal of two equivalent Me groups at δ 1·67 ppm and an isolated ABX-type signal must be due to the C_5H_9 residue; the signal of the ABX-type (δ 6·30, 4·93, and 4·87 ppm; J_{AX} = 17·6, J_{BX} = 10·6, and $|J_{AB}|$ = 1·2 Hz) can result from a vinyl group linked to a carbon atom having no hydrogen. Therefore, the structure of the C_5H_9 fragment was assigned as a 1,1-dimethylallyl group. The NMR parameters obtained are listed in Table 1.

The 100 MHz NMR spectrum of tetrahydroponcitrin in $CDCl_3$, shown in Fig 2b, differs from that of poncitrin as follows. The signal due to the *gem*-Me_2 of the 1,1-dimethylallyl group shifted upfield (δ 1·60 ppm), and the ABX-type signal due to the vinyl group disappeared, being replaced by an A_3B_2-type signal at δ 0·69 and 1·95 ppm, which must arise from an isolated Et group. This indicates that the 1,1-dimethylallyl group in poncitrin was reduced to a 1,1-dimethylpropyl group.

TABLE 2. NUCLEAR OVERHAUSER EFFECTS (%)[a] AND CHEMICAL SHIFTS (δ, PPM DOWNFIELD FROM TMS)[b] OBSERVED FOR TETRAHYDROPONCITRIN (XV) IN $CDCl_3$

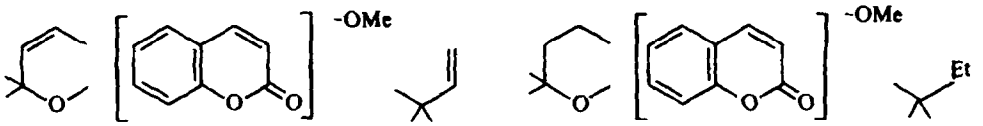
Saturated signals	Observed signals					
	3-H	4-H	6-H	7-H	12-H	13-H
	δ 6·15	7·90	2·81	1·77	1·95	0·69
	(5·97)	(7·47)	(2·52)	(1·33)	(2·13)	(0·85)
5-OMe	−5	11	2	0	0	0
δ 3·81						
(3·32)	(−6	(12)	(2)	(0)	(0)	(0)
8-Me_2	0	0	0			
δ 1·37						
(1·08)	(0)	(0)	(0)			
11-Me_2	0	0	0			
δ 1·60						
(1·80)	(0)	(0)	(0)			

[a] Increases in signal heights are shown in parentheses.
[b] Figures in parentheses are those obtained in C_6D_6.

Further, an isolated A_2B_2-type signal appeared at δ 1·77 and 2·81 ppm instead of the two doublets at δ 6·57 and 5·69 ppm corresponding to the olefinic protons in 2,2-dimethylcromene ring of poncitrin. The two doublets at δ 6·15 and 7·90 ppm (J = 9·7 Hz) due to the olefinic protons of the coumarin ring still remain. Thus, it was clear that the 2,2-dimethylchromene ring in poncitrin was reduced to a 2,2-dimethylchroman ring. Table 2 summarizes the NMR data.

These results led us to partial structures (I) for poncitrin and (II) for tetrahydroponcitrin. However, there are twelve possibilities for assemblage of the three substituents on the benzene ring in I, as represented in structures (III)-(XIX).* The correct structure for poncitrin was established by the use of NOE.[4]

* Arrows in the structural formulae indicate NOE's to be expected.



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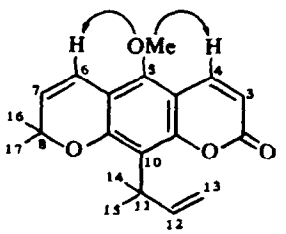
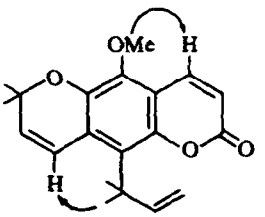


III



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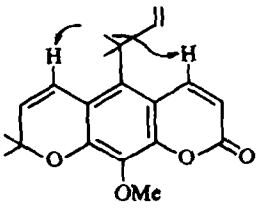

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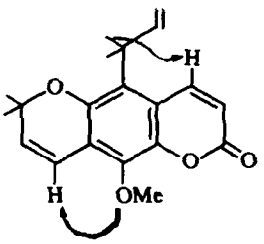

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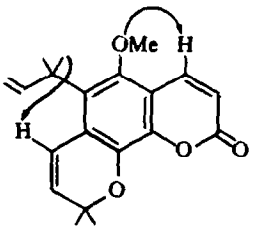

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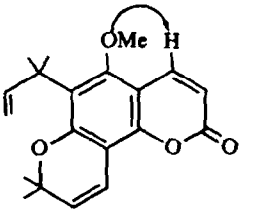
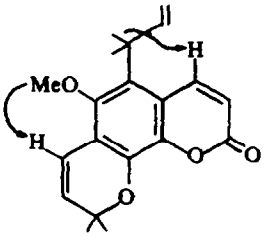


VIII



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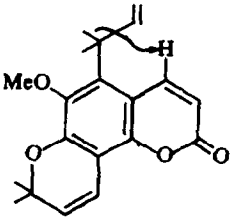
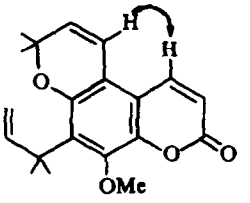
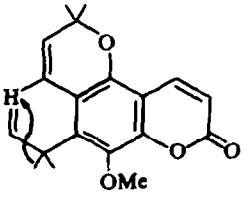


X



XI



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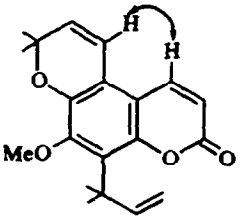
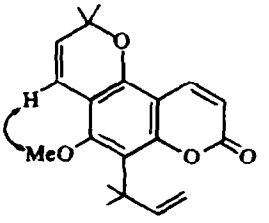
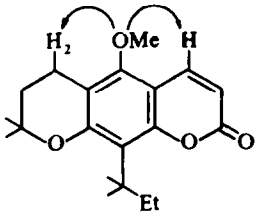


XIII

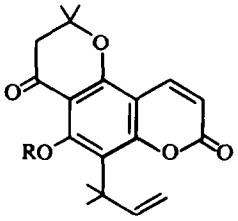


XIV

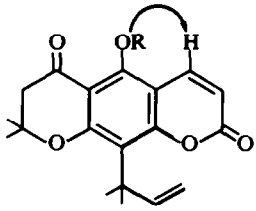
CHART I.



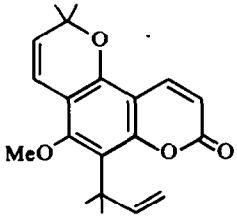
XV



XVI: R = H



XVII: R = H
XVIII: R = Me



XIX

CHART 2.

Thus, the signals of the OMe and the two *gem*-Me_2's in the spectra of poncitrin and tetrahydroponcitrin were successively saturated by double irradiation, and changes in the integrated intensities of the other proton signals were observed. When long-range spin-couplings were hardly detected between saturated and observed signals, changes in the signal heights were also recorded. The results of NOE experiments are also summarized in Tables 1 and 2.

On saturation of the OMe signals, the intensities of both olefinic 4-H and 6-H in poncitrin and those of both olefinic 4-H and aliphatic 6-H in tetrahydroponcitrin were appreciably increased. From these results, it was definitely concluded that the OMe group in poncitrin must be proximate to both 4-H and 6-H. Out of the twelve possible structures (III-XIV), only structure III meets this condition. Thus III should be the correct structure for poncitrin. Further, the NOE experiments also confirmed the presence of the 2,2-dimethylchromene and 1,1,-dimethylallyl group of poncitrin, and the NMR assignment of their *gem*-Me_2 groups.

It may be of interest that the NOE values obtained between vicinal protons in the double bonds are rather smaller than those expected from the short internuclear distance between them.[6] The effect of scalar coupling should be taken into account in these cases. The negative NOE values seen in Tables 1 and 2 are reasonable for the situation.[7]

The NMR spectra of poncitrin and tetrahydroponcitrin were also examined in the data being listed in Tables 1 and 2. The benzene-induced shifts[8] are quite consistent with the structures determined above; the characteristic lower-field shifts of the signals due to 11-Me, 12-H, and 13-H from $CDCl_3$ to C_6D_6 for both compounds demonstrate that these substituents are situated at the back side of the lactone grouping.

In conclusion, poncitrin and tetrahydroponcitrin have been determined as represented by structures III and XV, respectively. The 1,1-dimethylallyl side chain has so far been reported only in a few natural coumarins.[9,10] According to the present method,[2] Fuhrer *et al.*[11] recently reinvestigated the structures of clausenidin (XVI) and dentatin (XIX) to revise the former from (XVI) to (XVII) and to identify the latter to poncitrin; they found the NOE between the OMe group and 4-H in the methyl ether (XVIII) of clausenidin and obtained the same results with those presented here in dentatin.

EXPERIMENTAL

M.ps are uncorrected. NMR spectra were determined with a Varian HA-100 and an A-60A spectrometer by using *ca.* 10% (w/v) degassed solutions in $CDCl_3$ and C_6D_6 containing *ca.* 1% of TMS as an internal standard. Nuclear Overhauser effect experiments were performed on the HA-100 spectrometer in the frequency-swept and TMS-locked mode with a Hewlett-Packard HP-200ABR audiooscillater and an HP-5212A electronic counter, using sweep rates of 1 Hz per sec for integrations and 0·2 Hz per sec for signal heights. Accuracies are ±0·01 ppm for chemical shifts, ±0·2 Hz for coupling constants, and about ±2% for NOE. Mass spectra were measured with a Hitachi RMU-6E spectrometer.

Isolation of poncitrin from Poncirus trifoliata. The roots of *Poncirus trifoliata* RAFINESQUE were obtained from a hedge growing in School of Medicine, University of Tokushima. Cut and air-dried roots, 3·1 Kg, were extracted continuously for 3 days with boiling MeOH. The MeOH extract was concentrated under reduced pressure to a dark semi-solid concentrate. The syrupy residue was further extracted with boiling C_6H_6 for several days, and the extract was filtered. The total C_6H_6 solution was concentrated and then chromatographed on a column of neutral alumina. The bulk of the elution gave fractions containing mostly poncitrin. The solvent was evaporated to dryness to yield 2.22 g of colourless crystalline residue.

Poncitrin (III). Recrystallization from EtOH yielded colourless pillars, m.p. 93–94°; $[\alpha]_D^{14}$ ± 0° (*c* = 0·5, $CHCl_3$); λ_{max}^{EtOH} nm (log ε): 266 (4·40), 274 (4.45), 331 (4·06), 351 (4·05); ν_{max}^{KBr}: 1720 (C=O), 1608 (C=C), 1582 (aromatic C=C) cm^{-1}; NMR, see Table 1; Mass spectrum, *m/e*: 326 (M^+), 311 (M^+-Me) (base peak). (Found: C, 73·75, 73·71; H, 6·89, 6·79; OMe, 8·96 (Vieböck-Brecher method). Calcd. for $C_{20}H_{22}O_4$: C, 73·60; H, 6·79; OMe, 9·50%). The coumarin character of poncitrin was suggested by the fluorescence under filtered ultraviolet light. Its double-bond character was also recognized by a $KMnO_4$ solution test.

Tetrahydroponcitrin (XV). A solution of poncitrin (300 mg) in AcOH (30 ml) was added to a suspension of prereduced $PdCl_2$ on carbon (1% Pd) (300 mg) in the same solvent (5 ml), and hydrogenated at room temp and under atm. press. After 2 moles of hydrogen were absorbed (2 hr), the catalyst was filtered off, and the solution was neutralized with NaOH. The neutral solution was extracted exhaustively with Et_2O to remove Et_2O-soluble substances, and the ethereal extract was dried with $MgSO_4$. The solvent was evaporated, leaving a residue (260 mg), which was crystallized from EtOH to give colourless prisms, m.p. 94–95°; λ_{max}^{EtOH} nm (log ε): 256 (3·78), 264 (3·80), 338 (4·25); ν_{max}^{KBr} 1720 (C=O), 1610 (C=C), 1590 (aromatic C=C); NMR, see Table 2: Mass spectrum, *m/e*: 330 (M^+), 245 (base peak). (Found: C, 72·94; H, 8·00. Calcd. for $C_{20}H_{26}O_4$: C, 72·70; H, 7·93%).

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