Tetrahedron. 1967, Vol. 23, pp. 2523 to 2528. Pergamon Press Ltd. Printed in Northern Ireland

A NEW COUMARIN FROM GRAPEFRUIT PEEL OIL

J. F. FISHER and H. E. NORDBY
U.S. Fruit and Vegetable Products Laboratory,* Winter Haven, Florida 33880

and

A. C. WAISS, JR. and W. L. STANLEY
Western Regional Research Laboratory,† Albany, California

(*Received in USA* 23 *July* 1966; *accepted for publication* 25 *October* 1966)

Abstract—7-[(6′,7′-dihydroxy-3′,7′-dimethyl-2′-octenyl)oxy] coumarin has been isolated from the peel oil of grapefruit (*Citrus paradisi*). NMR, IR, UV, mass spectral and analytical data together with chemical data are presented as evidence of its constitution.

DURING an investigation on the composition of peel oil from grapefruit (*Citrus paradisi*), a hydroxylated coumarin (I) was isolated by column chromatography.[1] After recrystallization from ethyl acetate, compound I melted at 124–125° with $[\alpha]_D^{28} = +27°$. The elemental analysis of I agrees with a molecular formula of $C_{19}H_{24}O_5$. The OH determination showed that I contained two OH groups. The UV absorption spectrum of I is virtually identical with that of 7-geranyloxycoumarin[2,3] and is unchanged by the addition of base, indicating the absence of a free phenolic OH group. The IR absorption spectrum of I discloses bands at 3550 (m), 3400 (m), 1725 (s), and 1615 (s) cm^{-1}. The first two bands are in the OH region while the latter two are indicative of the coumarin lactone ring and an aromatic ring, respectively. A mol wt of 332 is obtained by mass spectrometry.[4]

The NMR[5] spectrum of I in $CDCl_3$ (Fig. 1) discloses signals at 6·25 (doublet, C-3 proton, J = 9·5 c/s) and 7·65 ppm (doublet, C-4 proton, J = 9·5 c/s), as expected of coumarin compounds.[1,6,7a] Signals at 7·38 (doublet, C-5 proton, J = 9·0 c/s) and 6·88 ppm (multiplet, C-6 and C-8 protons) are typical of the aromatic protons of a 7-alkoxyl substituted coumarin.[7b] A signal at 4·62 ppm (doublet, J = 6·5 c/s, 2H) is assigned to the methylene protons at C–1′. These protons occur at 4·64 ppm in

---

* A laboratory of the Southern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

† A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

[1] J. F. Fisher and H. E. Nordby, *J. Food Sci.* **30**, 869 (1965).

[2] W. L. Stanley, Recent developments in coumarin chemistry. *Aspects of Plant Phenolic Chemistry*. Proc. Third Ann. Symp. P.P.G.N.A., Toronto (1963).

[3] W. L. Stanley and S. H. Vannier, *J. Assoc. Off. Agr. Chemists* **40**, 582 (1957).

[4] The mass spectrum was obtained with a Bendix (TOF) mass spectrometer, model 12-100. The source was operated at 70 ev and recorded on an EAI X-Y Variplotter.

[5] All NMR spectra were obtained in $CDCl_3$ with a Varian A-60 spectrometer (unless otherwise stated) using TMS as an internal reference. Chemical shifts are in δ (ppm) values.

[6] W. L. Stanley, A. C. Waiss, Jr., R. E. Lundin and S. H. Vannier, *Tetrahedron* **21**, 89 (1965).

[7] [a] N. S. Bhacca, L. F. Johnson and J. N. Shoolery, *NMR Spectra Catalog* Vol. 1, Spectra Nos. 225 and 323. Varian Associates, Palo Alto, California (1962); [b] See spectrum no. 294; [c] See spectrum no. 279; [d] See spectra Nos. 44 and 310, also in Vol. 2, see Nos. 423 and 647; [e] See spectra Nos. 139, 142, 298, 363 and 366.

7-geranyloxycoumarin.[1] At 60 mc the vinyl proton at C-2′ appears at 5·53 ppm[1,7c] (triplet, J = 6·8 c/s, 1H). At 100 mc this absorption appears as a triplet (J = 6·8 c/s) with each peak further split into quartets (J = 1·2 c/s). This splitting and coupling constant along with stereochemical considerations and the reported trans arrangement of the Me group to hydrogen in geraniol[8] suggests the vinyl proton is *trans* to the Me group in I. Attempts to observe the split in the Me group failed. The signal at 1·78 ppm (singlet, 3H) is assigned to the vinyl Me group at C-3′. This chemical shift is identical to that of the vinyl Me group (1·78 ppm) in 7-geranyloxycoumarin[1] and similar vinyl Me groups.[9,10] The two methylene groups C-4′ and C-5′, appear at 2·30 ppm (multiplet, 4H). The signal at 3·38 ppm (multiplet, 1H) is assigned to the proton attached at C-6′. The two terminal Me groups appear as singlets at 1·18 and 1·22 ppm (total 6H). This constitutes an upfield shift of about 0·48 ppm over that seen for the terminal Me groups in the isobutylene system.[1,7c,9] This upfield shift is viewed as the result of the lower deshielding effect of the OH group in relation to a C=C double bond.[7d] The Me groups of isobutylene glycol appear at 1·16 ppm in deuterated acetone.[11] Protons in place of the OH groups would have an even greater diamagnetic influence on these Me groups.[7e] The different shielding values for the two terminal Me groups of compound I suggests that C-6′ is asymmetric as the case would be if I is a 6′,7′diol. A signal could not be observed for the OH groups of I in CDCl$_3$. However, when the NMR spectrum of I in deuterated DMSO was obtained,[12] a broad signal centered at 4·18 ppm (2H) appeared (Fig. 2). This signal is assigned to the OH groups since it disappeared on the addition of D$_2$O to the solution. The NMR spectrum of 7-geranyloxycoumarin in deuterated DMSO is devoid of signals in the region 3·5–4·5 ppm. Also, treatment of I with acetic anhydride and potassium acetate gives a product, the NMR spectrum of which in CDCl$_3$ shows one acetate peak at 2·12 ppm and a paramagnetic shift of about 1·36 ppm for the C-6′ proton.

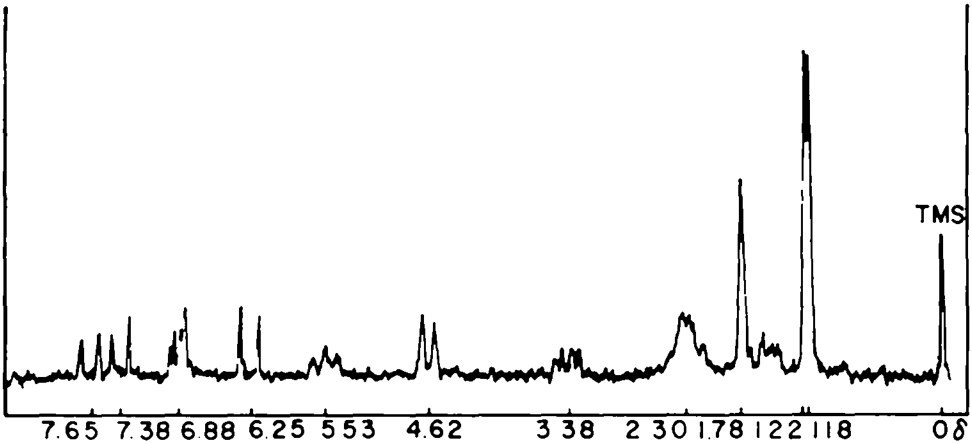


FIG. 1. NMR spectrum of compound I in CDCl$_3$.

---

[8] A. R. Pinder, *The Chemistry of the Terpenes* p. 35. Wiley, New York (1960).

[9] S. A. Francis and E. D. Archer, *Analyt. Chem.* 35, 1363 (1963).

[10] R. A. Flath, R. E. Lundin and R. Teranishi, *Tetrahedron Letters* No. 3, 295 (1966).

[11] J. F. Fisher and H. E. Nordby, Unpublished work.

[12] O. L. Chapman and R. W. King, *J. Am. Chem. Soc.* 86, 1256 (1964).

This order of shift is typical of acylation of secondary alcohols.[13] The t-OH group is well known to acetylate with difficulty, if at all.

In addition to the small parent peak at *m/e* 332 (5%), which is a reflection of the facile fragmentation of the allylic aliphatic side chain,[1,14] prominent ions are recorded at *m/e* 274, 171, 162, 153, 135, 134, 111, 81, 71, 59 and 43.

The partial fragmentation of I is shown in Fig. 3. The ion a, *m/e* 171 (21%) is the result of the facile fission of the allylic side chain between C-1′ and the ether oxygen[14] of compound I. This fragmentation with hydrogen rearrangement also produced ion b, *m/e* 162 which forms the base peak. The ready loss of CO from this α-pyrone system gives the benzofuran type ion c,[14] *m/e* 134 (26%). The ion d, *m/e* 153 (91%) is formed by the removal of a water molecule from ion a, *m/e* 171 while the loss of two molecules of water from ion a, *m/e* 171 gives ion e, *m/e* 135 (20%). Fragmentation of the vicinal glycolic ion a, *m/e* 171 between C-5′ and C-6′ followed by removal of a hydrogen atom from the hydrocarbon fragment and dehydration of the glycol portion[15] gives ions f, *m/e* 81 (47%) and an ion such as g, *m/e* 71 (66%), respectively. Cleavage between the 1,2-glycol structure of ion a results in the oxonium ion h, *m/e* 59 (46%) and a fragment which after the loss of a hydrogen atom forms ion i, *m/e* 111 (20%).[15] The ion j, *m/e* 274 (11%) and again ion h, *m/e* 59 is explained by fragmentation between the 1,2-glycol structure with hydrogen rearrangement of compound I.[15,16] This fragmentation pattern is characteristic of an isobutylene glycol.[16] The loss of a Me group and a hydrogen atom from ion h, *m/e* 59 gives the stable acetyl ion k, *m/e* 43 (95%).[15] The absence of a *m/e* 69 ion $Me_2—C=CH—CH_2^+$ is consistent with a 1,2-glycol arrangement at the C-6′ and C-7′ positions of compound I. This *m/e* 69 ion accounts for the characteristic base peak in the fragmentation of geraniol and similar terpenes.[17]

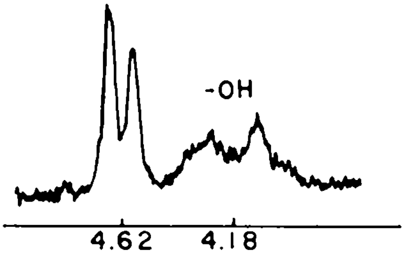


FIG. 2. Partial NMR spectrum of compound I in deuterated DMSO, showing a broad hydroxyl proton absorption at 4·18 δ relative to TMS at 0.

The fluorescence excitation and emission spectra[18] shows λ_{ex}^{EtOH} 352 mμ and λ_{em}^{EtOH} 390 mμ; these values are in the range obtained for similarly constituted coumarins.[1,19]

---

[13] L. M. Jackman, *Applications of NMR Spectroscopy in Organic Chemistry* p. 55. Pergamon Press, New York (1959).

[14] C. S. Barnes and J. L. Occolowitz, *Austr. J. Chem.* 17, 975 (1964).

[15] H. Budzikiewicz, C. Djerassi and D. H. Williams, *Interpretation of Mass Spectra of Organic Compounds*. Holden-Day, San Francisco (1964).

[16] F. A. Long and J. G. Pritchard, *J. Am. Chem. Soc.* 78, 2663 (1956).

[17] E. von Sydow, *Acta Chem. Scand.* 17, 2504 (1963).

[18] Fluorescence spectra were obtained with an Aminco-Keirs Spectrophosphorimeter.

[19] J. F. Fisher and H. E. Nordby, *Tetrahedron* 22, 1489 (1966).

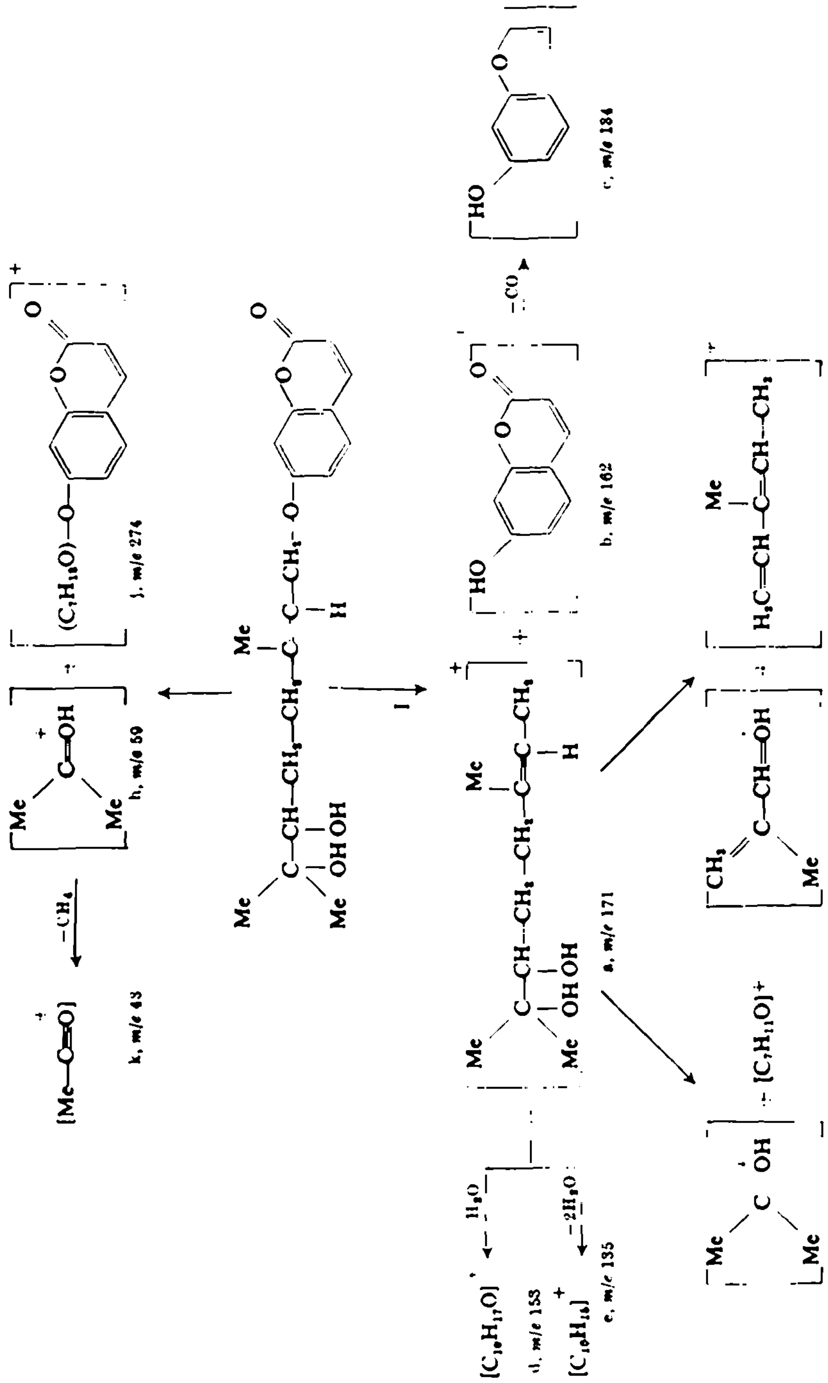
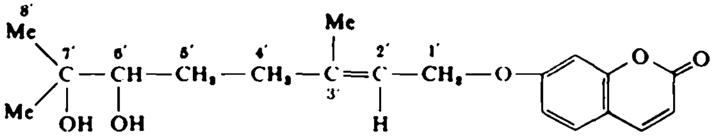


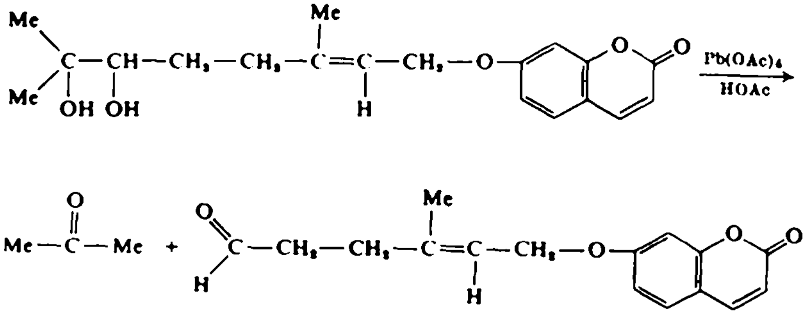
FIG. 3. Partial fragmentation of I.

The sum of the physical evidence indicates the constitution of I to be 7-[(6′,7′-dihydroxy-3′,7′-dimethyl-2′-octenyl)oxy] coumarin.



I

The above conclusion is confirmed by the following chemical evidence. Compound I gives a negative magnesium–hydrochloric acid test for flavonoids. Mild acid hydrolysis of I gives 7-hydroxycoumarin, a reaction which is characteristic of an allylic ether substituent at C-7 of coumarin.[2] Lead tetraacetate oxidation of I gives acetone and the aldehyde II. The aldehyde was confirmed by elemental analysis, IR, UV, NMR and its 2,4-DNPH derivative.



II

EXPERIMENTAL

Isolation of 7-[(6′,7′-*dihydroxy*-3′,7′-*dimethyl*-2′-*octenyl*)*oxy*] *coumarin* (I).

Cold-pressed Florida grapefruit peel oil[30] (5 gals) was concentrated, extracted, chromatographed and the fractions analysed by TLC.[1] The chromatographic fraction numbered 191[1] was concentrated *in vacuo*. The residue was recrystallized first from EtOH and then from AcOEt giving compound I as crystals, m.p. 124–125° and $[\alpha]_D^{24}$ = +27°. (Found: C, 68·95; H, 7·27; OH, 10·13. $C_{19}H_{24}O_5$ requires: C, 68·65; H, 7·28; OH, 10·22%.) The IR spectrum displayed bands at ν_{max}^{KBr} 3550(m), 3400(m), 1725(s), and 1615(s) cm^{-1}. The UV spectrum showed λ_{max}^{EtOH} 322 mμ (4·19), 253 mμ (3·42), and 243 mμ (3·61). The NMR spectrum showed signals at 6·25 (doublet, C-3 proton, J = 9·5 c/s), 7·65 (doublet, C-4 proton, J = 9·5 c/s), 7·38 (doublet, C-5 proton, J = 9·0 c/s), 6·88 (multiplet, C-6 and C-8 protons), 4·62 (doublet, J = 6·5 c/s, 2H), 5·53 (triplet, J = 6·5 c/s, 1H), 1·78 (singlet, 3H), 2·30 (multiplet, 4H), 3·38 (multiplet, 1H) and 1·18 and 1·22 ppm (two singlets, total 6H, two terminal Me groups). The mass spectrum showed prominent ions at *m/e* 274, 171, 162, 153, 135, 134, 111, 81, 71, 59, 43 and a mol wt of 332. The fluorescence excitation and emission spectra showed λ_{ex}^{EtOH} 352 mμ and λ_{em}^{EtOH} 390 mμ.

7-Hydroxycoumarin. A sample of I was dissolved in 1% ethanolic HCl and held at 40° for 3 hr. The soln was then evaporated under a stream of N_2. The residue was dissolved in MeOH and streaked on TLC plates of silica gel. The developing system was toluene, AcOEt, and AcOH (5:4:1). The band on each plate corresponding to authentic 7-hydroxycoumarin and showing a positive reaction with $FeCl_3$–$K_3Fe(CN)_6$ spray reagent (only a small portion of each plate was sprayed) was scraped

---

[30] We also found compound I in California grapefruit oil, see Ref. 2.

from the plates and eluted with EtOH. The recovered material, after evaporation of EtOH, was identified by a comparison of its UV spectrum and diagnostic bathochromic shift when treated with dil NaOH aq[21] as well as its R_f values in four different solvent systems with authentic 7-hydroxycoumarin.

Formation of acetone and the aldehyde (II). A soln of I (100 mg) and lead tetraacetate (150 mg) in AcOH (5 ml) was stirred overnight at room temp. Water (2 ml) was added and the soln evaporated *in vacuo*. Approximately 2 ml of distillate was collected in a liquid N_2 cooled trap. The distillate was treated with a 2,4-dinitrophenylhydrazine soln.[22] The resulting crude phenylhydrazone was chromatographed on silica gel (10 g). The column was developed by gradient elution of hexane with increasing amounts of AcOEt. The isolated 2,4-dinitrophenylhydrazone of acetone was identified by a comparison of its m.p., mixed m.p., IR and UV spectra with an authentic sample. The residue resulting from the vacuum distillation was diluted with water (10 ml), neutralized with $NaHCO_3$ and extracted with AcOEt (3 × 10 ml). The AcOEt extracts were combined, washed with water and dried over Na_2SO_4. Evaporation of the AcOEt *in vacuo* gave almost pure product (76 mg) m.p. 102·5°. Recrystallization from a mixture of AcOEt–hexane gave II as prisms, m.p. 104·6°. (Found: C, 70·4; H, 5·92. $C_{16}H_{16}O_4$ requires: C, 70·57; H, 5·92%.) The NMR spectrum of II between 3·5 and 8·0 ppm was essentially identical to that of I. The two terminal Me groups seen at 1·18 and 1·22 ppm in the spectrum of I were absent in II. The vinyl Me peak remained unchanged at 1·78 ppm. A signal in the NMR spectrum of II at 10·85 ppm (triplet, J = 1·3 c/s, 1H) was assigned to the aldehydic proton. The UV spectrum showed λ_{max}^{EtOH} 323 mμ (4·15). The IR spectrum displayed bands at $\nu_{max}^{CHCl_3}$ 2815(w), 2715(w), both characteristic of the CH stretching absorption in aldehydes[23] and 1725 cm^{-1}(s) C=O.

The 2,4-dinitrophenylhydrazone of II exhibited m.p. 153·5°. (Found: C, 58·5; H, 4·46; N, 12·3. $C_{22}H_{20}O_7N_4$ requires: C, 58·40; H, 4·46; N, 12·39%.)

References to specific products of commercial manufacture are for illustration only and do not constitute endorsement by the U.S. Department of Agriculture.

[21] F. M. Dean, *Naturally Occurring Oxygen Ring Compounds*. p. 177. Butterworth, London (1963).

[22] R. L. Shriner, R. C. Fuson and D. Y. Curtin, *The Systematic Identification of Organic Compounds* (5th edition) p. 219. Wiley, New York (1964).

[23] L. J. Bellamy, *The Infra-red Spectra of Complex Molecules* p. 156. Wiley, New York (1958).