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TWO NEW COUMARINS FROM GRAPEFRUIT PEEL OIL

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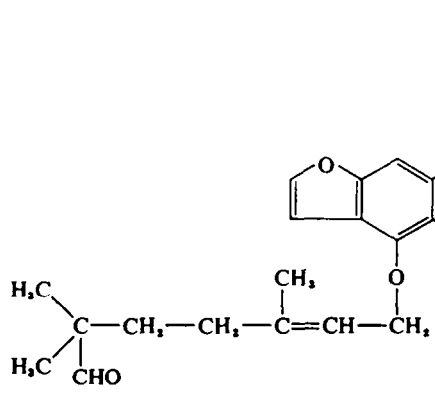
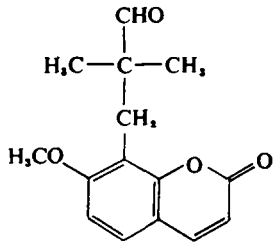
Abstract—5-[(3,6-dimethyl-6-formyl-2-heptenyl)oxy]-psoralen and 7-methoxy-8-(2-formyl-2-methylpropyl)-coumarin were isolated from grapefruit peel oil. NMR, IR, UV, mass spectral and analytical data are presented in support of their constitution.

DURING an investigation of the composition of grapefruit peel oil, compounds I and II were isolated by column chromatography in a manner similar to a published method.[2]



I



II

Formula I is proposed for the new psoralen and formula II for the new coumarin. This paper presents evidence to support the proposal that I is 5-[(3,6-dimethyl-6-formyl-2-heptenyl)oxy]-psoralen and II is 7-methoxy-8-(2-formyl-2-methylpropyl)-coumarin.

TLC on silica gel with rice starch as a binder,[3] in five different solvent systems indicated I was a pure compound.

Compound I, on TLC, showed a positive aldehyde reaction when sprayed with 2,4-dinitrophenylhydrazine.[4] This reaction was not observed with nonformylated psoralens. Compound I fluoresced yellow under UV light. The fluorescence excitation and emission spectra of I showed peaks at 348 mμ and 465 mμ, respectively.

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[2] W. L. Stanley and S. H. Vannier, *J. Amer. Chem. Soc.* **79**, 3488 (1957).

[3] L. L. Smith and T. Foell, *J. Chromatog.* **9**, 339 (1962).

[4] R. J. Block, E. L. Durrum and G. Zweig, *A Manual of Paper Chromatography and Paper Electrophoresis*. Academic Press, New York (1955).

Compound I melted from 134–136°. It showed a negative magnesium–hydrochloric acid test for flavonoid compounds. The elemental analysis of I agreed with a molecular formula of $C_{21}H_{22}O_5$. The UV spectrum of I was virtually superimposable on that of 5-geranyloxypsoralen.[2] Addition of base did not shift the UV spectrum of I, indicating the absence of a free phenolic hydroxyl group. The IR spectrum of I displayed bands at 2720 and 1730 cm^{-1}. In a compound containing only one carbonyl function these bands would be highly suggestive of an aldehyde.[5] However, since the coumarin lactone ring also absorbs in the region of 1730 cm^{-1} this band can only be used as evidence for the presence of a carbonyl group. Bands in the 1550–1630 cm^{-1} region are characteristic of an aromatic ring. A band at 1080 cm^{-1} is presumed due to a benzofuran structure.[6]

When compound I was warmed with aqueous methanolic KOH a yellow potassium salt was formed that relactonized on acidification. This reaction is characteristic of coumarin.[7] Mild acid hydrolysis of I gave 5-hydroxypsoralen, characteristic of an allylic ether substituent at C-5 of psoralen.[2]

Compound I displayed NMR[8] signals at 6·28 (doublet, C-3 proton, 1H, J = 10 c/s), 8·15 (doublet, C-4 proton, 1H, J = 10 c/s), 6·95 (doublet, C-6 proton, 1H, J = 2·5 c/s), 7·60 (doublet, C-7 proton, 1H, J = 2·5 c/s) and 7·14 (singlet, 1H, C-8 aromatic proton) all characteristic of a C-5 alkoxyl substituted psoralen.[9–11] The downfield shift of the C-4 proton to 8·15 is characteristic of a C-5 alkoxyl substituted psoralen.[9,10] The upfield position of the C-8 aromatic proton (7·14) is in keeping with the diamagnetic shift of an aromatic proton adjacent to an oxygen atom.[9,10,12,13] In psoralens a C-5 proton occurs at a lower field than the C-8 proton.[9,10] Additional NMR signals were observed at 4·96 (doublet, 2H, J = 6·5 c/s, methylene group adjacent to an oxygen atom), 5·60 (triplet, 1H, J = 6·5 c/s, vinyl proton at C-2 in the side chain), 1·72 (singlet, 3H, methyl group α to a double bond), 2·02 (unresolved, 4H, two methylene groups), 9·48 (singlet, 1H, aldehydic proton, formyl group attached to a quaternary carbon atom), and 1·10 (singlet, 6H, two terminal methyl groups). These signals are consistent with the proposed side chain moiety of compound I. The absence of splitting in the aldehydic proton signal (9·48) requires that the formyl group be attached to a quaternary carbon atom.

The mass fragmentation pattern of I showed prominent ions at *m/e* 202, 174 and 153. Fragmentation of the allylic side chain is readily accomplished under electron impact, thus, a parent peak was not obtained.[14] Fission of this side chain α to the ether oxygen with hydrogen rearrangement gave ion *m/e* 202 corresponding to the 5-hydroxypsoralen fragment and ion *m/e* 153 corresponding to the aliphatic aldehyde

[5] L. J. Bellamy, *The Infra-red Spectra of Complex Molecules*. J. Wiley, New York (1958).
[6] C. R. Ghoshal, S. S. Gupta and A. Chatterjee, *Chem. & Ind.* 1430 (1963).
[7] T. O. Soine, *J. Pharm. Sci.* **53**, 231 (1964).
[8] All NMR spectra were obtained in $CDCl_3$ with a Varian A-60 spectrometer using TMS as an internal reference. Chemical shifts are in δ values.
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[10] U. N. Sheinker, *Dokl. Akad. Nauk. SSSR* **158**, 1382 (1964).
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[12] J. B. Bredenberg and J. N. Shoolery, *Tetrahedron Letters* No. 9, 285 (1961).
[13] W. Bottomley, *Austr. J. Chem.* **16**, 143 (1963).
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fragment.[14] The facile loss of CO, characteristic of the α-pyrone ring,[14] gave ion *m/e* 174 from ion *m/e* 202.

TLC in five different solvent systems showed II to be a pure compound. Compound II, on TLC, displayed a positive aldehyde reaction when sprayed with 2,4-dinitrophenylhydrazine. This reaction was not observed with nonformylated coumarins.

Under UV light II showed a purple fluorescence. The fluorescence excitation and emission spectra displayed peaks at 352 mμ and 390 mμ, respectively. The bathochromic shift observed in the emission spectrum of I relative to that seen for II is ascribed to the furano structure of psoralens.[15] The UV spectrum of II was virtually identical with that of 7-methoxy-8-prenylcoumarin[16] (osthol) and was unchanged by the addition of base. The IR spectrum of II disclosed bands at 2820, 2720 and 1720 cm^{-1}. As in the case of compound I, the band at 1720 cm^{-1} can only be used as evidence for the presence of a carbonyl group. A band at 1590 cm^{-1} is characteristic of an aromatic ring.

Compound II melted from 137–138°. It showed a negative magnesium–hydrochloric acid test for flavonoid compounds. Compound II formed a yellow potassium salt that relactonized on acidification. Under acidic hydrolytic conditions II remained unchanged.

Compound II displayed NMR signals at 6·25 (doublet, C-3 proton, 1H, J = 9·5 c/s), 7·65 (doublet, C-4 proton, 1H, J = 9·5 c/s), 7·38 (doublet, 1H, J_{ortho} = 9·0 c/s, C-5 aromatic proton), 6·84 (doublet, 1H, J_{ortho} = 9·0 c/s, C-6 aromatic proton), 3·90 (singlet, 3H, methoxyl group at C-7), 3·10 (singlet, 2H, methylene protons at C-8), 1·10 (singlet, 6H, two equivalent methyl groups) and 9·60 (singlet, 1H, an aldehyde group attached to a quaternary carbon atom). The positioning of protons on carbon atoms 3,4,5 and 6, the methoxyl group on carbon-7, the five carbon side chain at carbon-8 and their NMR assignments are based on the following:

1. The chemical shifts assigned to the C-3 and C-4 protons are characteristic of coumarins.[9,11,13,17]

2. The chemical shift (7·65) assigned to the C-4 proton indicated the absence of an alkyl or alkoxyl substituent at C-5. Such a substituent at C-5 would result in a downfield shift of the C-4 proton to about 8.[9,18] Thus, the signal at 7·38 was assigned to a proton at C-5.

3. The spectrum disclosed a pair of doublets one at 7·38, J = 9·0 c/s assigned to the C-5 proton and the other at 6·84, J = 9·0 c/s which are typical of *ortho* protons in a 1,2,3,4 substituted benzene ring.[17,19] Therefore, the signal at 6·84 was assigned to a proton at C-6. The C-5 and C-6 proton assignments were further supported by the absence of a signal which could be assigned to a C-8 proton and the lack of C-6-mono-oxygenated coumarins in nature.[20]

4. The upfield shift (6·84) of the C-6 proton is characteristic of an aromatic proton adjacent to an oxygen substituent.[12,13] In view of the above and since nearly all

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[18] D. L. Dreyer, *Phytochem* in press.
[19] L. M. Jackman, *NMR Spectroscopy*. Pergamon Press, New York (1959).
[20] W. Karrer, *Konstitution und Vorkommen der Organischen Pflanzenstoffe* pp. 531–564, Birkhauser, Basel (1958); D. J. Austin and M. B. Meyers, *Phytochem.* **4**, 245 (1965).

naturally occurring coumarins possess an oxygen substituent at C-7,[21] the methoxyl group at 3·90 is considered to be at C-7.

5. Therefore, the methylene protons at 3·10[16] must be at C-8. Furthermore, the absence of splitting in this signal requires the methylene group to be attached to a quaternary carbon atom.

The chemical shifts observed for the protons on carbon atoms 3,4,5, and 6 and the methoxyl group located at C-7 were virtually identical with 7-methoxy-8-prenyl-coumarin.[9]

The mol. wt. of II as determined by mass spectrometry was 260. Other major ions were observed at *m/e* 232, 189, 159 and 131. The loss of CO from the parent ion gave ion *m/e* 232 with a benzofuran structure.[14] The base peak at *m/e* 189 was the tropylium-type ion resulting from fission of the side chain β to the aromatic ring.[14] This ion loses a CH_2O molecule giving ion *m/e* 159 followed by loss of CO to give ion *m/e* 131.[14,22] The absence of a M—CH_3 peak was consistent with the proposed structure of the five-carbon side chain.[14]

The sum of evidence is in favor of the formulations proposed for compounds I and II.

EXPERIMENTAL

Isolation of 5-[(3,6-*dimethyl*-6-*formyl*-2-*heptenyl*)*oxy*]-*psoralen* (I). A 5 gallon portion of cold pressed Florida grapefruit peel oil was concentrated, extracted, chromatographed and the fractions analysed on TLC as described earlier.[9] Chromatographic fractions numbered 128–136[9] showing a yellow fluorescence under UV light (3660 Å) were concentrated *in vacuo*. The residue was recrystallized from ethyl acetate–EtOH (1:1) giving I as crystals, m.p. 134–136°. (Found: C, 71·3; H, 6·24. $C_{21}H_{22}O_5$ requires: C, 71·2; H, 6·26%). The IR spectrum displayed bands at ν_{max}^{KBr} 2720 (w), 1730 (s), 1550–1630, and 1080 cm^{-1}. The UV spectrum showed λ_{max}^{EtOH} 307 mμ (4·28), 267 mμ (4·33), 258 mμ (4·35), 249 mμ (4·40) and 242 mμ (4·35). The fluorescence excitation and emission spectra[23] showed λ_{ex}^{EtOH} 348 mμ and λ_{em}^{EtOH} 465 mμ. The NMR spectrum showed signals at 6·28 (doublet, 1H, C-3 proton, J = 10 c/s), 8·15 (doublet, 1H, C-4 proton, J = 10 c/s), 6·95 (doublet, 1H, C-6 proton, J = 2·5 c/s), 7·60 (doublet, 1H, C-7 proton, J = 2·5 c/s), 7·14 (singlet, 1H, C-8 aromatic proton), 4·96 (doublet 2H, J = 6·5 c/s, methylene group adjacent to an oxygen atom), 5·60 (triplet, 1H, J = 6·5 c/s, vinyl proton at C-2 in the side chain), 1·72 (singlet, 3H, methyl group α to a double bond),[24] 2·02 (unresolved, 4H, two methylene groups),[25] 9·48 (singlet, 1H, aldehydic proton, formyl group attached to a quaternary carbon atom), and 1·10 (singlet, 6H, two terminal methyl groups). The mass spectrum showed prominent ions at *m/e* 202, 174, and 153. R_f values of 0·60 (ethyl acetate), 0·76 (toluene, ethyl acetate, acetic acid, 5:4:1), 0·94 (toluene, dioxan, water, upper layer 26°, 1:1:1), 0·22 (cyclohexane, ethyl acetate, 3:1) and 0·50 (tetrahydrofuran, hexane, 3:1) were obtained.

5-Hydroxypsoralen. A sample of I was dissolved in 1% ethanolic HCl and held at 50° for 3 hr. The solution was evaporated under a stream of N_2. The residue was dissolved in ethyl acetate and streaked on TLC of silica gel. The developing system was toluene, ethyl acetate and acetic acid (5:4:1). The band on each plate corresponding to authentic 5-hydroxypsoralen and showing a positive reaction with $FeCl_3$-$K_3Fe(CN)_6$ spray reagent[9] (only a small portion of the plate was sprayed) was scraped from the plates and eluted with EtOH. The recovered material, after evaporation of EtOH, was identified by a comparison of its IR and UV spectra as well as its R_f values in four different solvent systems with authentic 5-hydroxypsoralen.

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[22] N. S. Vul'fson, V. I. Zaretskii and V. G. Zaikin, *Bull. Acad. Sci. USSR Div. Chem. Sci.* 2046 (1963).
[23] All fluorescence spectra were obtained with an Aminco–Keirs Spectrophosphorimeter number 4-8201.
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[25] N. S. Bhacca, L. F. Johnson and J. N. Shoolery, *NMR Spectra Catalog*. spectrum No. 279. Varian Associates, Palo Alto, California (1962).

Chemical evidence for the coumarin moiety in I. Compound I was insoluble in 5% KOH aq but dissolved with a yellow color in warm 5% methanolic KOH aq. This solution remained clear when diluted with water but lost its color when acidified with HCl aq. The acidification regenerated the original compound as determined by TLC.

Isolation of 7-methoxy-8-(2-formyl-2-methylpropyl)-coumarin (II). The isolation of II was conducted in a manner similar to that employed for I. Chromatographic fractions numbered 159–167[9] showing a purple fluorescence under UV light (3660 Å) were concentrated *in vacuo*. The resulting residue was recrystallized twice (first from EtOH then from 3:1 hexane–ethyl acetate), giving II as fine white needles, m.p. 137–138°. The IR spectrum displayed bands at ν_{max}^{KBr} 2820 (w), 2720 (w), 1720 (s), and 1590 cm^{-1}. The UV spectrum showed λ_{max}^{EtOH} 320 mμ (4·10), 256 mμ (3·57) and 245 mμ (3·56). The fluorescence excitation and emission spectra showed λ_{ex}^{EtOH} 352 mμ and λ_{em}^{EtOH} 390 mμ. The NMR spectrum showed signals at 6·25 (doublet, 1H, C-3 proton, J = 9·5 c/s), 7·65 (doublet, 1H, C-4 proton, J = 9·5 c/s), 7·38 (doublet, 1H, J_{ortho} = 9·0 c/s, C-5 aromatic proton), 6·84 (doublet, 1H, J_{ortho} = 9·0 c/s, C-6 aromatic proton), 3·90 (singlet, 3H, methoxyl group at C-7), 3·10 (singlet, 2H, methylene protons at C-8), 1·10 (singlet, 6H, two equivalent methyl groups) and 9·60 (singlet, 1H, an aldehyde group attached to a quaternary carbon atom). The mass spectrum showed major ions at *m/e* 260, 232, 189, 159 and 131. R_f values of 0·41 (ethyl acetate), 0·50 (toluene, ethyl acetate, acetic acid, 5:4:1), 0·88 (toluene, dioxan, water, upper layer 26°, 1:1:1), 0·08 (cyclohexane, ethyl acetate, 3:1), and 0·39 (tetrahydrofuran, hexane, 3:1) were obtained.

Chemical evidence for the coumarin moiety in II. This was conducted in a manner similar to I.

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

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