Structures of Lansiumarin-A, -B, -C, Three New Furocoumarins from *Clausena lansium*

Chihiro ITO,* Shinya KATSUNO, and Hiroshi FURUKAWA

Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468, Japan.

Received July 24, 1997; accepted September 17, 1997

Three new furocoumarins named lansiumarin-A (1), -B (2), and -C (6) were isolated from the branches of *Clausena lansium* (Rutaceae), and their structures were elucidated by chemical and spectroscopic methods.

Key words *Clausena lansium*; furocoumarin; lansiumarin; hydroperoxide; Rutaceae

During our investigation of the constituents of plants of the genus *Clausena*,[1,2] various carbazoles and coumarins have been isolated and their structures determined. This paper describes the isolation of three new furocoumarins, lansiumarin-A, -B, and -C, from the branches of *Clausena lansium* (LOUR.) SKEELS, and the elucidation of their structures.

Results and Discussion

Dried branches of *C. lansium* were extracted with acetone at room temperature. The acetone extract was fractionated by a combination of silica gel column chromatography and preparative TLC to give three new furocoumarins along with some known coumarins.

Structure of Lansiumarin-A (1) Lansiumarin-A (1) was obtained (0.0007% yield) as a pale yellow oil. The molecular formula was determined as $C_{21}H_{20}O_5$ by high-resolution (HR)-MS. The UV spectrum [λ_{max}: 206 (sh), 218, 243 (sh), 248, 264 (sh), 300 nm] was similar to those of (*E*,*E*)-8-(7-hydroxy-3,7-dimethylocta-2,5-dienyloxy)psoralen (3) and (*E*)-8-(6-hydroperoxy-3,7-dimethylocta-2,7-dienyloxy)psoralen (5),[3,4] which were also isolated by us from the same plant. In addition, MS fragmentation[3] revealed a major ion at *m/z* 202 $[C_{11}H_6O_4]^+$ and IR bands at ν_{max} 1726 cm^{-1} (an α,β-unsaturated lactone), indicating the presence of an 8-oxygenated linear furocoumarin chromophore. The ^{1}H-NMR spectrum showed two characteristic pairs of doublets at δ 7.77, 6.37 (each 1H, $J=9.5$ Hz) assignable to H-4, H-3 on a coumarin nucleus and δ 7.69, 6.82 (each 1H, $J=2.2$ Hz) assignable to H-3′, H-2′ on a furan ring, as well as a lone singlet at δ 7.37 (H-5). Observations of the signals [δ_H 5.01 (2H, d, $J=7.0$ Hz); δ_C 69.96 (t), δ_H 5.61 (1H, m); δ_C 119.78 (d), δ_C 142.01 (s), δ_H 1.73 (3H, s); δ_C 16.80 (q), δ_H 2.31 (2H, t, $J=8.4$ Hz); δ_C 33.77 (t), δ_H 2.76 (2H, t, $J=8.4$ Hz); δ_C 35.71 (t), δ_C 201.24 (s, C=O), δ_C 144.36, δ_H 5.95 (1H, br s), 5.75 (1H, d, $J=1.5$ Hz); δ_C 124.59 (t), δ_H 1.86 (3H, s); δ_C 17.62 (q)] in the ^{1}H- and ^{13}C-NMR spectra, together with an IR absorption due to an α,β-unsaturated carbonyl group at ν_{max} 1676 cm^{-1}, indicated the presence of the side chain [$-OCH_2CH=C(CH_3)-CH_2CH_2-C(=O)-CCH_3(=CH_2)$] in the molecule. In nuclear Overhauser effect (NOE) expriments, irradiation of the methyl group at δ 1.73 resulted in 5% area increase of the signal at δ 5.01, suggesting the *E*-configuration of the double bond on the side chain. Further, the structure of 1 was confirmed by ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) spectroscopy, as shown by arrows in Fig. 1. Based on these spectral data, the structure of lansiumarin-A was concluded to be **1**.

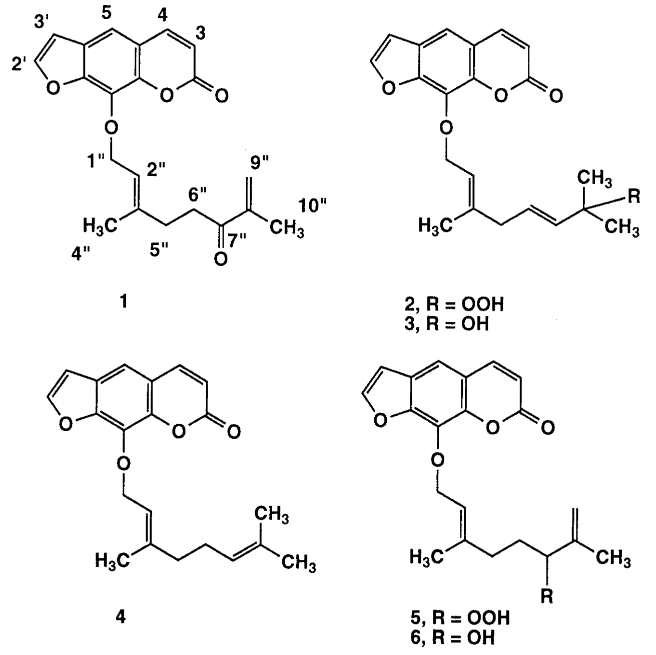

Chart 1

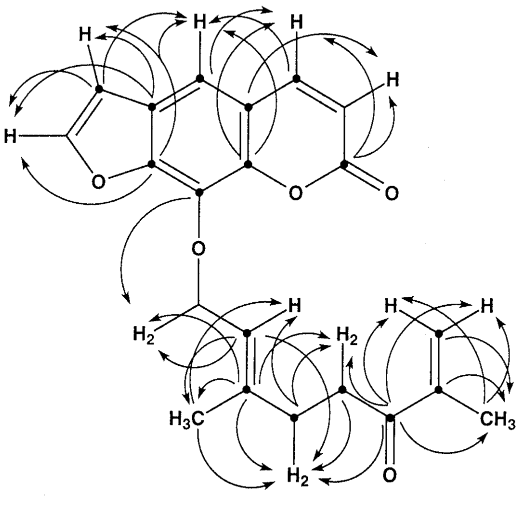

Fig. 1. C–H Long-Range Correlations in the HMBC Spectrum of Lansiumarin-A (1)

Structure of Lansiumarin-B (2) Lansiumarin-B (2) was obtained in 0.0029% yield as a pale yellow oil. The

---

* To whom correspondence should be addressed.

Table 1. ^{1}H-NMR Data for the New Furocoumarins in $CDCl_3$

		1	2	3	5	6
Coumarin skeleton	3-H	6.37 (d, J 9.5)	6.38 (d, J 9.5)	6.37 (d, J 9.5) .	6.37 (d, J 9.5)	6.37 (d, J 9.5)
	4-H	7.77 (d, J 9.5)	7.77 (d, J 9.5)	7.77 (d, J 9.5)	7.77 (d, J 9.5)	7.76 (d, J 9.5)
	5-H	7.37 (s)	7.38 (s)	7.37 (s)	7.37 (s)	7.36 (s)
Furan ring	2'-H	7.69 (d, J 2.2)	7.70 (d, J 2.2)	7.70 (d, J 2.2)	7.69 (d, J 2.2)	7.69 (d, J 2.2)
	3'-H	6.82 (d, J 2.2)	6.82 (d, J 2.2)	6.82 (d, J 2.2)	6.82 (d, J 2.2)	6.81 (d, J 2.2)
Side chain	1''-H	5.01 (2H, d, J 7.0)	5.02 (2H, d, J 7.6)	5.02 (2H, d, J 7.6)	5.03 (2H, d, J 7.3)	5.03 (2H, m)
	2''-H	5.61 (m)	5.65 (m)	5.63 (m)	5.64 (m)	5.63 (m)
	4''-H	1.73 (3H, s)	1.66 (3H, s)	1.68 (3H, s)	1.70 (3H, s)	1.69 (3H, s)
	5''-H	2.31 (2H, t, J 8.4)	2.73 (2H, d, J 6.3)	2.70 (2H, d, J 6.3)	2.05 (2H, t, J 6.8)	2.06 (2H, m)
	6''-H	2.76 (2H, t, J 8.4)	5.54 (m)	5.54 (m)	1.64 (2H, m)	1.58 (2H, m)
	7''-H	—	5.54 (m)	5.54 (m)	4.23 (t, J 6.8)	3.93 (t, J 5.9)
	9''-H	5.95 (br s) 5.75 (d, J 1.5)	1.31 (3H, s)	1.29 (3H, s)	5.00 (br s) 4.94 (br s)	4.87 (br s) 4.81 (d, J 1.5)
	10''-H	1.86 (3H, s)	1.31 (3H, s)	1.29 (3H, s)	1.71 (3H, s)	1.73 (3H, s)
	OOH	—	7.58 (br s)	—	7.96 (br s)	—

Values in (δ) ppm. The coupling constants (J) in parentheses are in Hz. All signals correspond to ^{1}H, unless otherwise stated.

molecular formula $C_{21}H_{22}O_6$ was presumed on the basis of the FAB-MS and ^{1}H-NMR spectra. The presence of an 8-oxygenated linear furocoumarin nucleus in this molecule was suggested by the appearance in the ^{1}H-NMR spectrum of two pairs of AB-type doublets [δ 7.77, 6.38 (each 1H, d, J=9.5 Hz), δ 7.70, 6.82 (each 1H, d, J= 2.2 Hz)] and a lone singlet at δ 7.38 due to H-5, together with UV and IR absorption bands (see Experimental). Further, the ^{1}H-NMR spectrum showed two 2H-doublets at δ 5.02 and 2.73, a 1H-multiplet at δ 5.65, a 3H-singlet at δ 1.66 due to a vinyl methyl, two 1H-multiplets at δ 5.54 due to vinyl protons, and a 6H-singlet at δ 1.31 due to two methyls attached to a carbon atom bearing an oxygen function. A signal at δ 7.58 was assigned to a hydroperoxy group. These spectral data, coupled with the observation of a significant mass fragment peak[3] at m/z 202, showed the structure [$-OCH_2CH=C(CH_3)-CH_2CH=CHC(CH_3)_2(OOH)$] for the side chain at C_8. For confirmation of the structure, the following chemical reaction was carried out. The hematoporphyrin-sensitized photo-oxygenation[5] of 8-geranoxypsoralen (**4**)[6,7] in oxygen gas gave two isometric peroxygenated products. One of them was found to be identical with natural **2** and the other with natural **5** by IR, ^{1}H-NMR, and co-TLC comparisons. These results comfirmed the structure of lansiumarin-B to be **2**.

Structure of Lansiumarin-C (6) Lansiumarin-C (**6**) was isolated (0.0005% yield) as a pale yellow oil having $[\alpha]_D$ $+8°$ ($CHCl_3$), $C_{21}H_{22}O_5$. The appearance of the characteristic signals [δ 7.76, 6.37 (each 1H, d, J=9.5 Hz), δ 7.69, 6.81 (each 1H, d, J=2.2 Hz)] and δ 7.36 (1H, s)] in the ^{1}H-NMR spectrum, together with the UV and IR bands (see Experimental), suggested that this compound possesses the same 8-oxygenated linear furocoumarin nucleus as **1** and **2**. Further, the remaining signals (see Table 1) in the ^{1}H-NMR spectrum revealed the presence of a C_{10} moiety [$-OCH_2CH=C(CH_3)-CH_2CH_2-CH(OH)C(CH_3)=CH_2$]. Treatment of **5**[3] with triphenylphosphine in MeOH at room temperature gave a colorless oil, which was found to be identical with natural **6** by IR and ^{1}H-NMR comparisons, and co-TLC. These results led us to propose the structure **6** for lansiumarin-C.

Experimental

^{1}H- and ^{13}C-NMR, NOE, 1H-detected heteronuclear multiple quantum coherence (HMQC), and HMBC (J=8 Hz) spectra were recorded on an A-400 or A-600 (JEOL) spectrometer, in $CDCl_3$. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference. All mass spectra were taken under electron impact (EI) conditions, unless otherwise stated, using an M-80 (Hitachi) spectrometer having a direct inlet system. UV spectra were recorded on a UVIDEC-610C double-beam spectrophotometer (Jasco) in MeOH, IR spectra on an IR-230 (Jasco) in $CHCl_3$, and optical rotations on a DIP-370 (Jasco) in $CHCl_3$ at 25 °C. Preparative TLC was done on Kieselgel 60 F_{254} (Merck).

Extraction and Isolation The dried branches (205 g) of *Clausena lansium* (LOUR.) SKEELS cultivated at Tokokuzan Fruit Garden (Nagoya) were collected in June, 1996, and extracted with acetone at room temperature. The acetone extract (3.91 g) was subjected to silica gel column chromatography eluted with hexane–acetone (19:1, 9:1, 17:3, 4:1, 7:3, 3:2, 2:3, 1:4), acetone, $CHCl_3$–MeOH (3:1), MeOH, successively, to give 11 fractions. The hexane–acetone (19:1) eluate was further subjected to silica gel column chromatography and preparative TLC with appropriate combinations of hexane, CH_2Cl_2, iso-Pr_2O, benzene, $CHCl_3$, EtOAc, acetone, MeOH as developing solvents to give three new furocoumarins, lansiumarin-A (**1**) (1.5 mg), -B (**2**) (6.0 mg), and -C (**6**) (1.0 mg), along with known coumarins.[8]

Lansiumarin-A (**1**): Pale yellow oil. UV λ_{max}: 206 (sh), 218, 243 (sh), 248, 264 (sh), 300 nm. IR ν_{max}: 1726, 1676, 1626, 1587 cm^{-1}. EI-MS m/z (%): 352 (M^+, 3), 202 (92), 187 (6), 173 (81), 157 (10), 151 (100). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 201.24 (s, C-7''), 160.46 (s, C-2), 148.71 (s, C-7), 146.63 (d, C-2'), 144.36 (s, C-8''), 144.32 (d, C-4), 143.93 (s, C-8a), 142.01 (s, C-3''), 131.52 (s, C-8), 125.84 (s, C-6), 124.59 (d, C-9''), 119.78 (d, C-2''), 116.47 (s, C-4a), 114.73 (d, C-3), 113.34 (d, C-5), 106.74 (d, C-3'), 69.96 (t, C-1''), 35.71 (t, C-6''), 33.77 (t, C-5''), 17.62 (q, C-10''), 16.80 (q, C-4''). HR-MS Calcd for $C_{21}H_{20}O_5$: 352.1308. Found: 352.1287.

Lansiumarin-B (**2**): Pale yellow oil. UV λ_{max}: 207, 217 (sh), 243, 248 (sh), 263 (sh), 296 nm. IR ν_{max}: 3500 (br), 1728, 1587 cm^{-1}. EI-MS m/z (%): 202 (100), 174 (82), 152 (29), 146 (20), 135 (63). FAB-MS m/z :393 $[M+Na]^+$.

Lansiumarin-C (**6**): Pale yellow oil. $[\alpha]_D$ $+8°$ (c=0.059). UV λ_{max}: 207, 218, 242 (sh), 249, 265 (sh), 299 nm. IR ν_{max}: 3490 (br), 1726, 1587 cm^{-1}. EI-MS m/z (%): 354 (M^+, 1), 202 (100), 174 (81), 157 (6), 151 (31). HR-MS Calcd for $C_{21}H_{22}O_5$: 354.1465. Found: 354.1436.

Photo-Oxygenation of 8-Geranoxypsoralen (4) Oxygen gas was bubbled through a solution of **4** (11.2 mg) in pyridine (6 ml) containing hematoporphyrin (2 mg), and the solution was irradiated with a high-pressure Hg lamp using a Pyrex glass filter for 0.5 h. Then, the solvent was evaporated off. The residue was subjected to silica gel preparative TLC [hexane : acetone (7:3)] to afford 3.1 and 1.4 mg of **2** and **5**, respectively. Compounds **2** and **5** were found to be identical with natural furocoumarins **2** and **5**, respectively, by spectrometric comparisons.

(*E*)-8-(6-Hydroperoxy-3,7-dimethylocta-2,7-dienyloxy)psoralen (**5**): Pale yellow oil. UV λ_{max}: 206 (sh), 218, 242 (sh), 249, 264 (sh), 299 nm. IR ν_{max}: 3523, 3350 (br), 1726, 1624, 1587 cm^{-1}. FAB-MS *m*/*z* :393 $[M+Na]^+$, 371 $[M+H]^+$.

Treatment of Natural Furocoumarin 5 with Triphenylphosphine A methanolic solution (0.5 ml) of **5** (1 mg) and Ph_3P (1 mg) was stirred overnight at room temperature. The solvent was evaporated off. The residue was subjected to preparative TLC [hexane : acetone (3 : 1)] to afford a colorless oil (**6**) (0.8 mg). This was found to be identical with natural furocoumarin **6** by comparisons of the IR and ^{1}H-NMR data.

Acknowledgements We are grateful to the staff of Tokokuzan Fruit Garden in Nagoya for supplying the plant material (*Clausena lansium*). This work was supported in part by a Grant-in-Aid (H. F., 1997) for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

References and Notes

1) Ito C., Ohta H., Tan H. T.-W., Furukawa H., *Chem. Pharm. Bull.*, **44**, 2231—2235 (1996).
2) Ito C., Katsuno S., Ohta H., Omura M., Kajiura I., Furukawa H., *Chem. Pharm. Bull.*, **45**, 48—52 (1997).
3) Rashid M. A., Gray A. I., Waterman P. G., *J. Nat. Prod.*, **55**, 851—858 (1992).
4) Murray R. D. H., Mendez J., Brown S. A., "The Natural Coumarins. Occurrence, Chemistry and Biochemistry," John Wiley & Sons Ltd., New York, 1982, pp. 27—51.
5) Murray R. D. H., Forbes I. T., *Tetrahedron*, **34**, 1411—1414 (1978).
6) Stanley W. L., Vannier S. H., *J. Am. Chem. Soc.*, **79**, 3488—3491 (1957).
7) Sharma Y. N., Zaman A., Kidwai A. R., Bates R. B., Thalacker V. P., *Tetrahedron*, **22**, 3221—3225 (1966).
8) Isolation and characterization of known coumarins isolated from this plant will be reported elsewhere.