Tetrahedron Letters No. 1, pp 7 - 10, 1975. Pergamon Press. Printed in Great Britain.

CARBON-13 NMR SPECTRA OF URS-12-ENES AND APPLICATION TO STRUCTURAL ASSIGNMENTS OF COMPONENTS OF ISODON JAPONICUS HARA TISSUE CULTURES

S. Seo, Y. Tomita, and K. Tori*

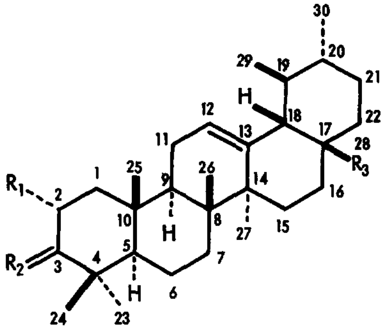
Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka, 553 Japan

(Received in Japan 6 November 1974; received in UK for publication 19 November 1974)

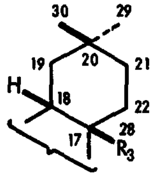
Olean-12-enes and urs-12-enes, triterpenes widely distributed in the plant kingdom, have frequently been isolated as mutually isomeric mixtures.[1] Recently, Doddrell, et al.[2] reported ^{13}C NMR spectroscopy as the most precise tool for the purpose of their distinction which is not easy between the two types.[1] However, their ^{13}C signal assignments remain incomplete. In a previous paper,[3] we then reported complete assignments of ^{13}C NMR signals of several olean-12-enes to aim the more powerful use for structure assignments and biosynthetic studies. Here, we further report our full signal assignments in ^{1}H-noise-decoupled natural abundance ^{13}C FT NMR spectra of several urs-12-enes (1-6) together with those of their corresponding olean-12-enes (7-12) and their application to structural assignments of components synthesised by the callus derived from Isodon japonicus H.[4]

The ^{13}C signals were assigned by means of single-frequency off-resonance decoupling techniques,[3,5] by applications of known chemical-shift rules such as hydroxyl substitution and acetylation shifts, and steric γ and δ effects,[5,6] and from comparisons of the spectra from compound to compound, including those of the olean-12-enes treated in the previous study.[3] In addition of these techniques, line-broadenings of CH_2 and CH resonances of these types of compounds were useful for diagnosis, as described previously.[3] This kind of line-broadenings probably due to molecular association were not observed for the spectra of (1) and (7), and occurred slightly in those of (5) and (11), these observations are in conformity with their cause.[3] The Table lists the ^{13}C chemical shift data, δ_C, determined.

Inspection of the Table revealed some interesting δ_C differences between an isomeric pair of an urs-12-ene and an olean-12-ene, besides those in δ_C values for olefinic C-12 and C-13 already explained by Doddrell, et al.[2] Considerably large δ_C differences between two series were discernible for the C-18 (ca.



Urs-12-enes



The corresponding olean-12-enes

		R_1	R_2	R_3		
α-amyrin	(1)	H	$<^{\mathrm{OH}}_{\cdots\mathrm{H}}$	Me	(7)	β-amyrin
	(2)	H	$<^{\mathrm{OAc}}_{\cdots\mathrm{H}}$	Me	(8)	
methyl ursolate	(3)	H	$<^{\mathrm{OH}}_{\cdots\mathrm{H}}$	CO_2Me	(9)	methyl oleanolate
	(4)	H	$<^{\mathrm{OAc}}_{\cdots\mathrm{H}}$	CO_2Me	(10)	
	(5)	H	=O	CO_2Me	(11)	
	(6)	OH	$<^{\mathrm{OH}}_{\cdots\mathrm{H}}$	CO_2Me	(12)	methyl maslinate
		OH	$<^{\mathrm{H}}_{\cdots\mathrm{OH}}$	CO_2Me	(13)	methyl 3-epi-maslinate

12 ppm), C-19 (ca. 7 ppm), and C-20 (ca. 8.5 ppm) signals, although all δ_C values for the carbons concerning ring E should be changed on going from an oleanene to its isomeric ursene. Unexpectedly, the C-11 and C-27 signals in the ursene series were found to appear at higher fields by about 6.0 and 2.7 ppm, respectively, than those in the oleanene series (see the Table). These differences can be explained by a conformational change in the E ring caused by the spatially close proximity of the C-29 methyl group with C-12, C-13, and C-27 in ursenes. A twisted form of the E ring was shown by smaller $J_{H-18,H-19\alpha}$ values (10.7 Hz) observed in the 100-MHz ^1H NMR spectra of the ursenes than those (13.6 Hz) in the spectra of the oleanenes in $CDCl_3$ δ(H-18) and δ(H-19) are 2.21 (doublet) and 2.83 (quartet) for (3) and (9), respectively. The conformational change in the E ring is accompanied with those in the C and D rings, accordingly, these changes may also be responsible for the differences.

Recently, we reported in a biosynthetic study of triterpenes that the callus derived from Isodon japonicus

TABLE. Carbon-13 Chemical Shifts (δ_C) of Urs-12-enes (1-6) and the Corresponding Olean-12-enes (7-13) (in Parentheses) in $CDCl_3$ [a]

Carbon	(1)	(7)	(2)	(8)	(3)	(9)^{b}	(4)	(10)^{b}	(5)	(11)	(6)	(12)	(13)
C-1	38.7	(38.5)	38.4	(38.2)	38.8	(38.5)	38.3	(38.1)	39.3	(39.1)	46.8	(46.4)	(47.7)
C-2	27.2	(27.0)	23.6	(23.6)	27.3	(27.1)	23.6	(23.6)	33.9	(34.2)	68.9	(68.8)	(66.5)
C-3	78.8	(78.9)	80.7	(80.7)	78.8	(78.7)	80.7	(80.7)	216.5	(216.8)	83.8	(83.8)	(78.9)
C-4	38.7	(38.7)	37.6	(37.6)	38.8	(38.7)	37.6	(37.5)	47.1	(47.3)	39.1	(39.1)	(38.5)^{c}
C-5	55.2	(55.1)	55.3	(55.3)	55.4	(55.2)	55.3	(55.2)	55.2	(55.2)	55.4	(55.3)	(48.1)
C-6	18.3	(18.3)	18.3	(18.3)	18.4	(18.3)	18.1	(18.2)	19.5	(19.5)	18.4	(18.3)	(18.1)
C-7	32.9	(32.6)	32.8	(32.6)	33.0	(32.6)	32.8	(32.6)	32.4	(32.1)	32.9	(32.6)	(32.5)
C-8	40.0	(39.7)	40.1	(39.7)	39.6	(39.3)	39.5	(39.3)	39.1	(39.1)	39.6	(39.1)	(39.7)^{c}
C-9	47.7	(47.6)	47.6	(47.6)	47.5	(47.6)	47.4	(47.5)	46.6	(46.7)	47.5	(47.5)	(47.4)
C-10	36.9	(37.0)	36.8	(36.8)	37.0	(37.0)	36.8	(36.9)	36.6	(36.6)	38.3	(38.3)	(38.3)^{c}
C-11	17.4	(23.4)	17.5	(23.4)	16.9	(23.1)	17.1	(23.0)	16.8	(23.0)	17.0	(23.1)	(23.2)
C-12	124.3	(121.7)	124.1	(121.5)	125.5	(122.1)	125.4	(122.1)	125.0	(121.9)	125.3	(122.0)	(122.1)
C-13	139.3	(145.0)	139.4	(144.9)	138.0	(143.4)	138.0	(143.6)	137.9	(143.5)	138.1	(143.6)	(143.8)
C-14	42.0	(41.7)	42.1	(41.7)	42.0	(41.6)	41.9	(41.6)	41.9	(41.7)	42.1	(41.7)	(41.9)
C-15	28.7	(28.3)	28.7	(28.3)	28.2	(27.7)	28.1	(27.7)	27.9	(27.6)	28.0	(27.6)	(27.7)
C-16	26.6	(26.2)	26.7	(26.2)	24.3	(23.4)	24.2	(23.6)	24.1	(23.5)	24.3	(23.5)	(23.4)^{d}
C-17	33.7	(32.5)	33.8	(32.5)	48.1	(46.6)	48.0	(46.6)	47.9	(46.7)	48.1	(46.6)	(46.8)
C-18	58.9	(47.2)	59.0	(47.2)	52.8	(41.3)	52.8	(41.3)	52.8	(41.2)	52.8	(41.3)	(41.3)
C-19	39.6^{c}	(46.8)	39.7^{c}	(46.8)	39.1^{c}	(45.8)	38.9	(45.8)	38.8	(45.7)	39.1^{c}	(45.8)	(46.0)
C-20	39.6^{c}	(31.1)	39.7^{c}	(31.1)	38.8^{c}	(30.6)	38.9	(30.6)	38.8	(30.5)	38.9^{c}	(30.7)	(30.7)
C-21	31.2	(34.8)	31.3	(34.8)	30.7	(33.8)	30.7	(33.8)	30.5	(33.7)	30.7	(33.8)	(34.0)
C-22	41.5^{c}	(37.2)	41.5^{c}	(37.1)	36.7	(32.3)	36.6	(32.3)	36.6	(32.1)	36.7	(32.3)	(32.5)
C-23	28.1	(28.1)	28.1	(28.1)	28.2	(28.1)	28.1	(28.0)	26.5	(26.4)	28.7	(28.6)	(28.5)
C-24	15.6	(15.5)	16.8	(16.8)	15.5^{d}	(15.6)^{c}	16.9	(16.8)	21.3^{c}	(21.3)	17.0	(16.8)	(21.9)
C-25	15.6	(15.5)	15.7	(15.7)	15.7^{d}	(15.3)^{c}	15.5	(15.3)	15.1	(14.8)	17.0	(16.8)	(16.4)
C-26	16.8	(16.8)	16.8	(16.8)	16.9	(16.8)	16.9	(16.8)	16.8	(16.7)	17.0	(16.8)	(17.0)
C-27	23.3	(26.0)	23.2	(26.0)	23.3	(26.0)	23.2	(25.8)	23.4	(25.7)	23.4	(26.0)	(26.2)
C-28	28.1	(27.3)	28.1	(27.0)	177.7	(177.9)	177.6	(177.8)	177.3	(177.7)	177.9	(178.0)	(178.1)
C-29	23.3^{e}	(33.2)	23.2^{e}	(33.4)	23.6^{e}	(33.1)	23.6^{e}	(33.1)	23.4^{e}	(33.0)	23.7^{e}	(33.1)	(33.2)
C-30	21.3^{e}	(23.6)	21.4^{e}	(23.6)	21.2^{e}	(23.6)	21.2^{e}	(23.6)	21.1^{c,e}	(23.5)	21.2^{e}	(23.5)	(23.6)^{d}
CO_2Me	-		-		51.4	(51.3)	51.3	(51.4)	51.2	(51.3)	51.5	(51.5)	(51.5)
OCOMe			21.2	(21.2)			21.1	(21.2)					
OCOMe			170.4	(170.4)			170.5	(170.5)					

---

[a] ^{13}C FT NMR spectra were taken with a Varian NV-14 FT NMR spectrometer at 15.09 MHz. Samples were dissolved in $CDCl_3$ containing TMS as an internal standard (δ_C 0), concentrations were about 0.5-1 mmole/cm^3. FT NMR measurement conditions were as follows· spectral width 3319 Hz, pulse width. 12 µsec, acquisition time 0.6 sec, and number of data points. 4096. [b] Data taken from ref. 3. [c,d] Assignments may be reversed. [e] These assignments might be reversed, although those given here are considered to be valid.

H. retains ability to produce oleanolic, maslinic, and 3-epi-maslinic acids.[4] Thereafter, examinations of ^{13}C NMR spectra of the methylation products of each triterpene fraction have clearly revealed that the methyl oleanolate (9) and maslinate (12) fractions contain their corresponding ursene type isomers (3) and (6)[7] in ratios of about 1 . 1 and 7 · 5, respectively, whereas the methyl 3-epi-maslinate (13) fraction does not contain its isomer. The result suggests that epimerization[4] of a 3β-OH in the ursene derivative (6) does not take place in the callus owing to the conformational difference in the E ring between the oleanene and ursene skeletons. The signals due to C-11, C-18, C-19, and C-27 as well as those of C-12 and C-13 in the mixture spectra were of much dianostic value. The δ_C values for (13) are also included in the Table.

A study of biosynthesis of the triterpenes in the tissue cultures for proving Ruzicka's proposition[8] is now in progress by use of ^{13}C-enriched mevalonic acids.

Acknowledgements. We thank Prof. I. Kitagawa of Osaka University for providing us with samples of α- and β-amyrin acetates and Dr. Y. Takeuchi of this laboratory for a sample of ursolic acid.

REFERENCES

(1) For example, see R. Tschesche and G. Wulff, Progr. Chem. Org. Natural Products **30**, 461 (1973).

(2) D. M. Doddrell, P. W. Khong and K. G. Lewis, Tetrahedron Letters 2381 (1974).

(3) K. Tori, S. Seo, A. Shimaoka and Y. Tomita, Tetrahedron Letters, in press.

(4) Y. Tomita and S. Seo, J. C. S. Chem. Comm. 707 (1973).

(5) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," Wiley-Interscience, New York (1972), J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York (1972).

(6) S. H. Grover and J. B. Stothers, Can. J. Chem. **52**, 870 (1974).

(7) A. T. Glen, W. Lawrie, J. McLean and M. E.-G. Younes, J. Chem. Soc. (C) 510 (1967).

(8) L. Ruzicka, Experientia **9**, 357 (1953).