The ^{13}C Nuclear Magnetic Resonance Spectra of Kauranoid Diterpenes

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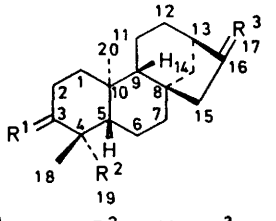
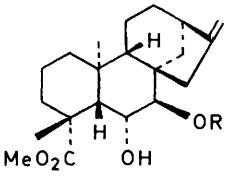
The ^{13}C n.m.r. spectra of some kauranoid diterpenes have been assigned. The application of the results to the determination of the sites of hydroxylation in this series is discussed.

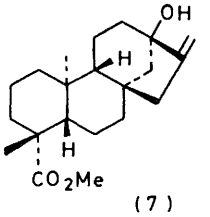
THE number of known naturally occurring tetracyclic kauranoid diterpenes is rapidly increasing.[1] These substances possess a common carbon skeleton but differ from another in hydroxylation pattern. If the ^{13}C n.m.r. signals of the parent *ent*-kaurene (1) can be assigned, then ^{13}C n.m.r. spectroscopy has a useful role to play in locating the sites of hydroxylation in new members of this series. This paper presents these assignments.



(1) $R^1 = H_2, R^2 = Me, R^3 = CH_2$
(2) $R^1 = H_2, R^2 = Me, R^3 = O$
(3) $R^1 = O, R^2 = Me, R^3 = O$
(4) $R^1 = H_2, R^2 = CO_2H, R^3 = O$
(9) $R^1 = H_2, R^2 = Me, R^3 = \alpha\text{-OH}, \beta\text{-Me}$



(5) R = H
(6) R = Ac



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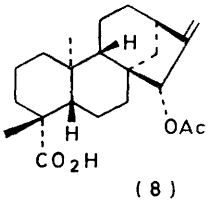


(8)

The spectra were determined at 25.15 MHz by using a pulsed Fourier transform system with proton noise-decoupling. The multiplicities of the resonances in the off-resonance-decoupled spectra were used to distinguish between methyl, methylene, methine, and quaternary carbon signals. Two series of compounds were examined. The first, (1)—(9), had structures based on that of *ent*-kaurene (1), and included oxidation products containing functionality on rings A, B, and D and at the CD ring junction. The second series (10)—(18) had structures

[1] J. R. Hanson, 'The Tetracyclic Diterpenes,' Pergamon, Oxford, 1968, ch. 2; 'Terpenoids and Steroids' ed. K. H. Overton, Chem. Soc. Specialist Periodical Reports, (*a*) 1971, vol. 1, p. 141; (*b*) 1972, vol. 2, p. 140; (*c*) 1973, vol. 3, p. 175; (*d*) 1974, vol. 4, p. 157.

^{13}C N.m.r. spectra of some kauranoid diterpenes [δ_C values (from internal Me_4Si)]

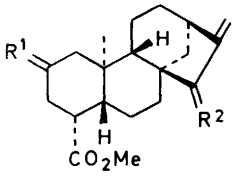
Comp.	Carbon atom																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	OCH$_3$
(1) [a]	41.3	18.7	42.0	33.3	56.1	20.3	40.4	44.2	56.1	39.3	18.1	33.3	44.2	39.9	49.2	156.0	102.8	33.7	21.7	17.6	
(2) [a]	41.0	18.5	41.9	33.2	56.1	19.2	40.3	42.5	55.0	39.4	18.5	29.7	47.9	37.5	55.2	222.5		33.6	21.7	18.0	
(3) [b]	39.0 *	33.9	217.1	47.0	53.6	20.3	39.9 *	42.2	54.2	38.5	19.0	29.3	47.6	36.9	54.6	221.5		27.3	21.0	18.1	
(4) [c]	41.0	18.7	37.6	43.7	53.9	20.7	40.6	42.4	56.7	39.7	18.7	29.5	47.7	37.3	54.9	222.7		28.9	184.2	16.1	
(5) [d]	38.9	19.6	38.9	46.1	49.0 *	70.9	79.0	46.3	51.4 *	38.7	18.1	33.4	43.8	42.5	45.4	154.3	103.3	28.9	181.4	18.5	52.8
(6) [d]	39.0	19.6	39.0	45.3	50.1 *	67.9	80.1	45.1	52.7 *	38.8	18.1	33.2	43.8	42.6	45.8	153.9	103.4	29.1	181.0	18.4	52.7 †
(7) [e]	40.6	19.1	38.0	43.7	53.8	21.8	39.2	41.6	56.9	39.2	20.4	41.3	80.1	46.9	47.4	155.9	102.9	28.6	177.9	15.2	51.1
(8) [f]	40.8	19.1	38.9	43.7	47.0	21.4	37.7	46.0	56.5	39.4	18.0	33.3	40.6	36.4	81.6	153.7	106.1	28.9	184.6	15.9	
(9) [a]	42.0	18.6	42.0	33.2	56.2	20.4	40.3	45.3	56.8	39.3	18.0	26.9	49.0	37.7	58.0	79.4	24.5	33.5	21.6	18.0	‡
(10) [g]	49.0	63.8	37.3	43.8	49.0	25.4	34.8	47.4	51.2	40.3	18.2	32.4	42.1	36.2	82.4	159.4	108.5		175.6	16.4	52.8
(11) [g]	48.2	65.8	39.1	43.9	48.7	22.7	33.7	47.3	51.4	39.8	18.2	32.3	42.0	36.5	82.6	159.6	108.7	175.8		16.5	51.4
(12) [g]	56.1	208.5	42.8	45.3	48.3	24.9	34.3	47.4	51.7	42.0	18.1	32.1	42.0	35.6	82.2	159.6	108.7		173.0	17.0	52.1
(13) [g]	55.2	208.9	43.6	45.1	47.7	23.0	33.2	47.7	50.4	42.0	18.1	32.1	42.0	35.8	82.2	159.3	108.8	174.9		16.9	52.0
(14) [g]	55.3	207.8	43.0	45.3	47.5	24.1	32.8	51.7	50.2	43.0	18.1	31.7	37.9	35.9	209.6	148.9	115.1		175.4	16.7	51.7
(15) [g]	48.3	64.0	37.4	43.7	48.3	24.4	33.2	52.2	50.8	40.8	18.2	32.0	38.0	36.5	210.3	149.1	114.9		175.3	16.2	51.4
(16) [h]	47.6	63.8	42.6	58.6	53.1	23.1	34.8	48.7	51.0	40.0	18.3	32.5	42.2	36.3	82.4	159.5	108.7	172.7	172.7	17.0	52.1
																					52.8
(17) [i]	49.9	202.2	53.4	42.3	48.2	24.2	34.1	47.3	51.6	43.3	18.0	32.0	41.9	35.6	82.0	159.2	108.8		171.5	16.7	52.1
(18) [i]	45.0	67.7	33.5	43.3	48.6	24.0	34.8	44.5	51.5	40.3	18.8	29.4	46.8	33.5	80.3	216.8			174.3	16.8	52.7 §

* Assignments may be interchanged. † Acetate 21.2 and 169.8. ‡ Acetate 171.4 and 21.4. § Acetates 20.6, 21.3, 169.9, and 170.1.

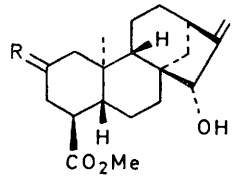
[a] L. H. Briggs, B. F. Cain, R. C. Cambie, B. R. Davis, P. S. Rutledge, and J. K. Wilmshurst, *J. Chem. Soc.*, 1963, 1345; J. R. Hanson, *ibid.*, p. 5061. [b] D. A. H. Taylor, *J. Chem. Soc.* (*C*), 1967, 1380. [c] C. A. Henrick and P. R. Jefferies, *Austral. J. Chem.*, 1964, **17**, 578. [d] B. E. Cross, R. H. B. Galt, and J. R. Hanson, *J. Chem. Soc.*, 1963, 2944. [e] E. Mosettig and W. R. Nes, *J. Org. Chem.*, 1955, **20**, 884. [f] D. E. Ekong and A. U. Ogan, *J. Chem. Soc.* (*C*), 1968, 311. [g] F. Piozzi, A. Quilico, R. Mondelli, T. Ajello, V. Sprio, and A. Melera, *Tetrahedron*, 1966, Suppl. 8, p. 515. [h] B. Danielli, R. Bombardelli, A. Bornati, and B. Gabetta, *Phytochemistry*, 1972, **11**, 3501. [i] G. Savonna, unpublished work.

based on that of atractyligenin (10).[2] The results are tabulated.

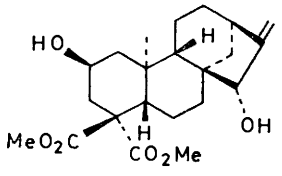
The three methyl groups of *ent*-kaurene (1) (C-18—20) were distinguished by the effect of the presence of a



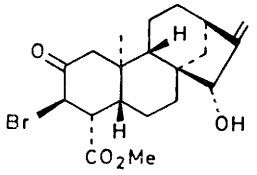
(10) R^1 = H, β-OH, R^2 = α-OH, H
(12) R^1 = O, R^2 = α-OH, H
(14) R^1 = R^2 = O
(15) R^1 = H, β-OH, R^2 = O



(11) R = H, β-OH
(13) R = O



(16)



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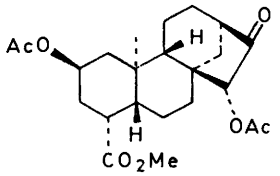

(18)

based on that of atractyligenin (10).[2] The results are tabulated.

The three methyl groups of *ent*-kaurene (1) (C-18—20) were distinguished by the effect of the presence of a 19-oic acid group (4); the signal associated with C-19 was then replaced by a low-field resonance at δ 184.2 and that associated with C-18 showed an upfield shift from δ 33.7 to 28.9. The assignment of the resonance at δ 17.6 to C-20 was supported by comparison with the atractyligenin series in which this is the sole methyl group. The resonances associated with carbon atoms 1, 2, 3, 6, 7, 11, 12, 14, 15, and 17 in *ent*-kaurene (1) appeared as triplets in the off-resonance spectrum. These include a group of three resonances around δ 20 and four between δ 40 and 42. The olefinic triplet associated with C-17 was readily recognized. The spectrum of *ent*-kaurene differed from that of *ent*-17-norkauran-16-one (2) in that a low field triplet in the former at δ 49.2 had moved to δ 55.2 in the latter. This signal, which was absent from the spectrum of xylopic acid (8), was assigned to C-15. In the spectrum of *ent*-17-norkaurane-3,16-dione (3) a triplet had moved downfield from δ 18.7 to 33.9 and a triplet at δ 42.0 had been replaced by a singlet at δ 217.1. These signals were assigned to C-2 and C-3. Base-catalysed deuteriation of *ent*-17-norkaurane-3,16-dione gave a [$^2H_4$]-species in which the ^{13}C resonances at δ 33.9 and 54.6 had collapsed, confirming the assignment of these to the enolisable centres, C-2 and C-15. In both the 17-nor-16-ketones a signal which appeared at δ 33.3 in the spectrum of *ent*-kaurene (1) had moved upfield to δ 29.7 in a manner characteristic of C-12 in the kaurenolides.[3] Another resonance had moved upfield from δ 39.9 to 37.5 and this was assigned to C-14. A signal at δ 41.3 in *ent*-kaurene had also moved upfield to 39.0 in the 3-ketone and this was assigned to C-1. The remaining triplet in the δ 40 group, which was unaffected by changes at C-3 and C-16 and yet was absent in the 6,7-diol (5), was assigned to C-7. Of the group of triplets around δ 20, that due to C-2 has already been assigned, a C-6 signal was absent in the case of the

[2] F. Piozzi, A. Quilico, R. Mondelli, T. Ajello, V. Sprio, and A. Melera, *Tetrahedron*, 1966, Suppl. 8, p. 515.
[3] J. R. Hanson, G. Savona, and M. Siverns, *J.C.S. Perkin I*, 1974, 2001.

6,7-diol, and the C-11 resonance was recognized from the effect of a 13-hydroxy-group in steviol methyl ester (7). A resonance at δ 17.8 had previously been assigned to C-11 in the kaurenolides.[3] In compounds (4)—(8), bearing a 19-oic acid or ester group, the C-3 resonance had moved upfield from δ 42 in *ent*-kaurene to δ 38—39. In steviol methyl ester (7) the resonance assigned to C-14 had moved downfield to δ 46.9 owing to the effect of the adjacent hydroxy-group. The position of this resonance also reflects substitution at C-7 [*cf.* (4) and (5)]. The overall grouping of the methylene ^{13}C resonances reveals the shielding effect of 1,3-diaxial interactions on C-2, C-6, and C-11.

The doublet resonances associated with C-5, C-9, and C-13 were distinguished by again comparing *ent*-kaurene (1) with the ketones (2) and (3). In the 16-ketone (2) the resonance associated with C-13 had moved downfield from δ 44.2 to 47.9 whereas that associated with C-9 showed a small upfield shift. The remaining resonance at δ 56.1 associated with C-5 had moved upfield in the spectrum of the 3-ketone (3). In the case of the 6,7-diol (5), the signals assigned to C-6 and C-7 were distinguished by the effect of acetylation [(5) ⟶ (6)]. The resonance associated with C-7 moved downfield and that associated with C-6 moved upfield as expected.[4]

Singlets were associated with C-4, C-8, C-10, and C-16. The olefinic resonance was easily recognized. A signal at δ 39, which remained relatively constant throughout the series, was assigned to C-10. The resonance at δ 33.3 in *ent*-kaurene (1), which moved downfield to δ 47.0 in the 3-ketone (3) and to 43.7 in the 19-oic acid (4), was assigned to C-4. The position of the resonance at δ 44.2, assigned to C-8, reflected changes both on ring D and at C-7.

The atractyligenins (10)—(18) were originally considered as part of the kaurene series. However a number of the resonances were sufficiently different in position to require independent assignment. Although the disposition of functionality differs between the series, these differences may also be partly steric in origin, arising from the absence of one of the C-4 substituents. Indeed the position of the C-6 and C-7 resonances lies between the values in the kaurene series (two C-4 substituents) and the steroids (no C-4 substituents, C-6 δ 29.2, C-7 δ 32.6).[5]

The triplet resonances associated with C-1 and C-3 in atractyligenin (10) were assigned on the basis of their change in the C-2 ketones (12)—(14). When the ketone (13) was deuteriated, the signals at δ 43.6 and 55.2 collapsed. The signal from C-3 was distinguished from that of C-1 by the effect of bromination at C-3 and by the effect of introducing a second methoxycarbonyl group at C-4 (16). The resonance at δ 25.4 was assigned to C-6 since its changes reflected epimerization at C-4. The resonances associated with C-11, C-12, and C-14 were assigned by comparison with the kaurene and kaurenolide[3] series. In particular in the nor-ketone (18), the resonances associated with C-12 and C-14 showed characteristic upfield shifts. The remaining triplet, which showed changes on variation of both the C-4 and C-15 substituents, was assigned to C-7.

The doublet resonances associated with C-4, C-5, C-9, and C-13 were readily assigned. Deuteriation of (13) led to the collapse of a signal at δ 45.1 which was therefore assigned to C-4. A doublet in the δ 47.5—49.0 region was assigned to C-5 since it reflected to a small extent the oxidation level of C-2 and epimerization at C-4 whereas it changed to a much larger extent in the presence of a second methoxycarbonyl group at C-4. The resonance assigned to C-9 remained relatively constant, reflecting in a minor way the changes in shape of the molecule arising from the epimerization at C-4 and the conversion of sp^3 centres into sp^2 centres. The resonance associated with C-13 was readily distinguished by the effect of a C-15 (15) and a C-16 (18) oxo-group. The singlet resonances associated with C-8 and C-10 were easily distinguished by the effect of an oxo-group at C-15 (10)—(15) or at C-2 (10)—(12), respectively.

In conclusion, it is possible to distinguish the sites of hydroxylation of a kaurene from the effect of the hydroxy-group both on the site of hydroxylation and on the adjacent carbon resonances. Not only are the methyl resonances distinct, but amongst the methylene resonances those associated with C-2, C-6, and C-11 can be distinguished from those assigned to C-1, C-3, C-7, and C-14, and these in turn can be distinguished from the resonances of C-12 and C-15. Thus if hydroxylation occurs at C-2, not only is this signal displaced but two triplets associated with the adjacent C-1 and C-3 will be shifted. If C-6 is hydroxylated then the C-5 (doublet) and C-7 (triplet) signals will also be shifted and similarly if C-11 is hydroxylated then the C-9 (doublet) and C-12 (δ 33 triplet) signals will be shifted.[3] Similar reasoning applies to the other triplets, and amongst the doublets C-13 is distinct from C-5 and C-9. Although there are some general similarities between the spectra of these compounds and those of the gibberellins[6] and the kaurenolides,[3] the presence of the lactone rings clearly introduces sufficient skeletal distortion to affect markedly the position of some of the signals. Some similar differences exist between the kaurene and stachene[7] series. Such limitations must be recognized when comparisons are made in the presence of functionality potentially capable of distorting the carbon skeleton.

EXPERIMENTAL

The ^{13}C n.m.r. spectra were determined with a JEOL PFT-100 Fourier transform spectrometer operating at 25.15 MHz (spectral width 6.25 kHz; 8 192 data points; 1—10 000 accumulations; pulse length 7 μs at a pulse interval of 1.0 s). The samples (80—150 mg) were dis-

[4] M. Christl, H. J. Reich, and J. D. Roberts, *J. Amer. Chem. Soc.*, 1971, **93**, 3463.
[5] H. Eggert and C. Djerassi, *J. Org. Chem.*, 1973, **38**, 3788.
[6] R. Evans, J. R. Hanson, and M. Siverns, *J.C.S. Perkin I*, 1975, 1514.
[7] J. R. Hanson and M. Siverns, unpublished results.

solved in [^{2}H]chloroform (0.5 ml). The solvent deuterium provided the lock signal. Tetramethylsilane was used as internal standard. Chemical shifts are estimated to be accurate to within ± 0.1 p.p.m.

Deuteriation of the 3,16-*Diketone* (3).—Sodium (100 mg) was dissolved in methan[^{2}H]ol (5 ml). The diketone (3) (150 mg), in a small amount of ether, was added and the solution was heated under reflux for 2 h and then left overnight at room temperature. The solution was acidified with [^{2}H]hydrochloric acid and diluted with water. The product [M^+ 292 ($C_{19}H_{24}{}^2H_4O_2$)] was recovered in ether. The exo-ester (13) was treated similarly at room temperature.

[5/1395 *Received*, 15th *July*, 1975]