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THE ^{13}C NMR SPECTRA OF SOME ROSANE DITERPENOIDS

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Key Word Index—*Trichothecium roseum*; fungi; biosynthesis; ^{13}C NMR, rosenonolactone.

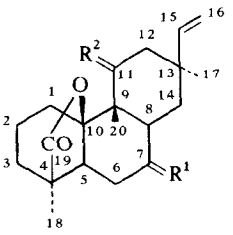
Abstract—The ^{13}C NMR signals of rosenonolactone have been assigned utilizing the ^{13}C-^{13}C couplings in material obtained biosynthetically from sodium [1,2-$^{13}C_2$] acetate.

In connection with biosynthetic work on the rosane metabolites of the fungus, *Trichothecium roseum* [1] we have assigned the ^{13}C NMR signals of this group of diterpenoid.

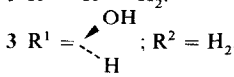
This information, which forms the subject of this report, may also have value in structural work in this series. The known diterpenoid rosane lactones include desoxyrosenonolactone (**1**), [2] rosenonolactone (**2**), [3] rosenololactone (**3**), [4] 11β-hydroxyrosenonolactone (**4**) [5] and rosoloactone (**5**) [6]. The ^{13}C NMR spectra of a number of tricyclic diterpenoids of the pimarane and abietane series have been assigned [7]. Conventionally ^{13}C NMR assignments have been made utilizing known functional group resonances and comparing changes between related compounds. However the labelling pattern of rosenonolactone (**2**), biosynthesized from [^{14}C] acetate and mevalonate, has been determined [8]. We have therefore used this information to assist in the assignments. In particular ^{13}C-^{13}C coupling constants derived from material biosynthetically labelled with [1,2-$^{13}C_2$] acetate, link pairs of atoms. In the case of the rosanes, particularly where there are differing multiplicities in the SFORD spectrum for members of the pairs, this serves to diminish the ambiguity of assignments.

Rosenonolactone (**2**), which is the most abundant rosane, was prepared biosynthetically from sodium [1,2-^{13}C] acetate and from sodium [1-^{13}C] acetate. The spectra of this diterpenoid and of compounds (**1**), (**3**) and (**5**) were determined in the noise-decoupled and single frequency off-resonance decoupled (SFORD) modes. The assignments and coupling constants are given in Table 1.

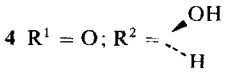
The resonances of rosenonolactone were assigned first. The singlet resonance which was associated with the quaternary carbon atom, C-4, showed a 48.8 Hz coupling to the lactone carbonyl, C-19, whilst the singlet assigned to C-13, showed a 36.6 Hz coupling to a methyl quartet associated with C-17 thus defining these pairs of resonances. The signals associated with the carbonyl groups and C-10 appeared within the accepted ranges. The remaining singlet, coupled to a triplet, was then assigned to C-9. The doublet resonances associated with the methine carbons, C-5 and C-8, were distinguished in the following manner. The doublet resonance at δ 50.6 was coupled to a triplet at δ 30.8 whilst the doublet resonance at δ 47.3 was coupled to another triplet at



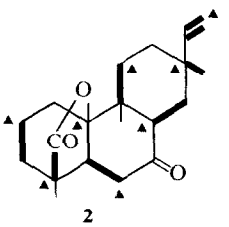
1 $R^1 = R^2 = H_2$.



3 R^1 = ; $R^2 = H_2$.



4 $R^1 = O$; R^2 =



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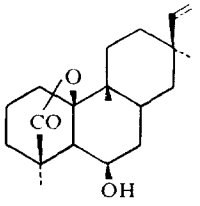
— anticipated ^{13}C–^{13}C couplings from [1,2-$^{13}C_2$] acetate

▲ positions enriched from [1-^{13}C] acetate.



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δ 31.7. When [1-^{13}C] acetate was fed to the fungus, the signal at δ 30.8 was enriched and hence this was assigned to C-6 and thus the resonance at δ 50.8 belonged to C-5. The other pair, in which the triplet resonance would not be expected to be enriched, was then assigned to C-8 and C-14. Typically the olefinic coupling between C-15 and C-16 was 70.2 Hz. Amongst the triplet resonances, the methylenes C-6, C-11 and C-14 were assigned on the basis of their couplings to C-5, C-9 and C-8 respectively, together with the appropriate enrichment of C-6 and C-11 from [1-^{13}C] acetate. The resonances associated with C-2 and C-3 were coupled (J = 32.1 Hz) and distinguished from one another by the enrichment of C-2 from [1-^{13}C]-acetate. The remaining triplet resonances (C-1 and C-11) were more difficult to assign with the material available. The suggested assignments are based on comparison with the gibberellin series [9]. Amongst the methyl groups that associated with C-17 was clear from the coupling patterns, whilst in rosenonolactone C-18 and C-20 were co-incident. The resonances of the other rosanes were then assigned by comparison with those of rosenonolac-

Table 1. ^{13}C NMR signals of rosanes in ppm from Me_4Si

Carbon atom	Compound (1)	(5)	(3)	(2)	Coupling constant in (2); Hz	% enrichment of (2), 1-^{13}C.
1	30.9*	31.1*	31.0*	30.2*		
2	19.9	19.9	19.9	19.8	32.1	0.33
3	36.0	36.4	35.6	35.4	32.1	
4	47.4	45.7	47.2	47.3	48.8	0.20
5	52.5	55.5	50.5	50.8	33.5	
6	18.2	63.5	29.2	30.8	33.5	0.25
7	25.7	37.4	69.1	210.1		
8	30.9	32.0	37.3	47.3	36.6	0.20‡
9	38.3	37.3	38.3	38.3	35.1	
10	88.2	87.8	88.0	87.0		0.39
11	29.7*	31.7*	34.0	35.8	35.1	0.24
12	32.2*	32.3*	31.9*	31.4*		
13	36.0	36.0	35.9	35.1	36.6	0.39
14	40.4	40.0	31.8	31.7	36.6	
15	150.5	150.5	150.4	149.6	70.2	
16	109.2	109.1	109.5	109.9	70.2	0.20
17	22.3	22.4	22.0	21.8	36.6	
18	14.7†	13.5†	17.2	16.9		
19	180.7	181.3	180.3	179.2	48.8	
20	17.0†	16.9†	17.2	16.9		

*† These assignments may be interchanged; ‡ overlapping signal; enrichment divided by 2

tone. The quaternary carbon resonances showed little change with substituent, C-4 showing a slight γ-shielding effect on the introduction of a hydroxyl group at C-6. The signal assigned to the C-5 methine carbon showed, as anticipated, a downfield shift on the introduction of the C-6 hydroxyl group in rosololactone (5) and a small upfield γ-shift on the introduction of the oxygen functions at C-7. On the other hand the C-7 substituents caused a considerable downfield shift of the C-8 resonance. The C-6 and C-7 methylene resonances in desoxyrosenonolactone were assigned on the basis of known substituent effects. The resonance assigned to C-14 showed a marked γ-shielding effect on the introduction of the oxygen functions at C-7.

The biosynthetic labelling patterns utilized in this work were fully in accord with the ^{14}C results. This method of assignment where the biosynthetic labelling patterns are known or obvious, may have value in diminishing ambiguity. Furthermore, bearing in mind the cost of instrument time and the effort involved in making suitable derivatives, it may be no more expensive than the conventional method of comparing a larger series of compounds.

EXPERIMENTAL

The ^{13}C NMR spectra were determined on a JEOL PFT 100 spectrometer operating at 25.15 MHz. The spectral width was 6.25 KHz; 8192 data points were used for 5–10 000 accumulations. The pulse length was 11 μsec at a pulse interval of 1 sec. The samples (20–50 mg) were dissolved in $CDCl_3$ (0.5 ml); Me_4Si was used as an int. stand. The coupling constants are accurate to ± 1.5 Hz.

Feeding experiments. Trichothecium roseum (CMI 50,660) was grown in shake culture on a medium as described previously [1]. [1,2-$^{13}C_2$] Na acetate (200 mg) containing Na [1-^{14}C] acetate (4 μCi) was distributed between 4 flasks (100 ml each) after 5 days growth. After 2 further days, the mycelium was filtered and disrupted by freezing with liq. N_2. It was extracted with Me_2CO–EtOAc (1:1) (150 ml). The extract was dried and the solvent evapd. The residue was washed with petrol and subjected to PLC in EtOAc–petrol (1:1) to afford rosenonolactone (23 mg), mp 213–214° (lit. [3], 214°) (0.42% incrop. ^{14}C), identified by comparison with an authentic sample. The above experiment was repeated with Na [1-^{13}C] acetate (200 mg) (4 μCi, ^{14}C) to afford rosenonolactone (22.3 mg) (0.34% incorp. ^{14}C).

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