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ASSIGNMENT OF THE ^{13}C NMR SPECTRA OF FUSIDIC ACID DERIVATIVES. BIOSYNTHETIC INCORPORATION OF SODIUM [1-^{13}C]-ACETATE INTO FUSIDIC ACID

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Fusidic acid (I) (1-5), a useful antibiotic in the treatment of staphylococcal infections in man, differs from the androstane and cholestane steroids by having the ring B forced into a boat conformation (6,7) and by having two extra methyl groups. Due to the unusual stereochemistry of (I), it may be expected that the results of ^{13}C NMR investigations on androstane and cholestane steroids (8,9) only have limited application to the fusidane ring system, since ^{13}C chemical shifts are profoundly influenced by stereochemical factors (10). We report here the complete assignment of all carbon signals in the ^{13}C NMR spectra of (I) and its methyl ester (II), their ^{1}H chemical shifts as determined from selective ^{13}C-$\{^{1}H\}$ spin decoupling experiments and finally, definite information on the biosynthesis of (I).

As shown in Figure 1, the carbon atoms in (I) and (II) give rise to separate signals in their ^{1}H noise decoupled ^{13}C spectra (0.10 m $CDCl_3$; 25.2 MHz; Varian XL-100-15 spectrometer, S124-XL Fourier transform accessory, Varian 620L 16K computer) except that the signals for C18 and C26 overlap*. The complete assignment of the signals to the individual carbon atoms in (II) (and (I)) was first of all rendered possible by comparison with the ^{13}C spectra of a series of methyl ester derivatives** including the esters of 3-epi-, 11-epi-, 3-keto-, 11-keto-, 3,11-diketo-, 7-α-hydroxy-, and 16-deacetyl-fusidic acid, thus permitting identification from already known substituent effects, empirical correlations and substituent parameters (10). Secondly, a series of selective ^{13}C-$\{^{1}H\}$ decoupling experiments for all the protonated carbon atoms in

* At lower solute concentration (0.04 m) for (I) this signal splits into a doublet.

** The use of methyl esters in the assignment studies was preferred since, at the concentration used, the acids give rise to dipole-dipole broadening in both the ^{1}H and ^{13}C spectra due to intermolecular associations (increase in correlation time for molecular reorientation).

each of these compounds established a very useful correlation between the ^{13}C and ^{1}H chemical shifts; furthermore, information regarding the number of protons bonded to the individual carbons was obtained from the splittings in the off-resonance decoupled carbon signals in these experiments. This decoupling technique also permitted a distinction between the two almost identical ester carbonyl signals by selectively decoupling the long-range ^{13}C-^{1}H couplings due to the widely separated methyl and methoxy protons. Finally, the small differences in ^{13}C shifts for (I) and (II) immediately gave the assignment of the ^{13}C

TABLE 1. ^{13}C and ^{1}H Chemical Shifts[a] of Fusidic Acid (I) and its Methyl Ester (II).

Carbon	δ_C^{TMS}				δ_H^{TMS}	
	(I)	(II)	(III)[b]	(IV)[c]	(I)	(II)
1	30.21	30.27	34.40	37.6	1.83	1.87
2	29.91	30.03	31.72	32.1	1.83	1.80
3	71.59	71.43	76.58	70.7	3.76	3.74
4	36.51	36.45	39.72	38.9	1.58	1.59
5	36.02	36.09	43.00	45.5	2.14	2.16
6	20.96	20.92	21.04	29.3	1.40	1.34
7	32.12	32.29	32.86	32.6	1.43	1.43
8	39.57	39.54	39.52	36.1	-	-
9	49.46	49.41	49.16	55.1	1.58	1.57
10	36.98	37.03	36.83	35.9	-	-
11	68.29	68.30	68.37	21.7	4.35	4.34
12	35.69	35.66	36.02	40.7	2.12	2.10
13	44.31	43.97	43.96	43.1	3.06	3.05
14	48.79	48.76	48.79	57.0	-	-
15	39.04	39.13	39.17	24.6	1.73	1.72
16	74.54	74.47	74.42	28.6	5.90	5.86
17	150.51	148.19	148.08	57.0	-	-
18	17.83	17.77	17.79	12.4	0.92	0.92
19	23.08	22.97	23.74	12.3	0.98	0.98
20	129.85	130.46	130.64	36.2	-	-
21	174.22	170.75	170.66	18.9	-	-
22	28.82	28.99	28.99	36.7	2.52	2.47
23	28.49	28.37	28.32	24.4	2.14	2.08
24	123.19	123.15	123.13	40.0	5.12	5.11
25	132.53	132.54	132.58	28.3	-	-
26	17.83	17.77	17.79	22.7	1.60	1.60
27	25.71	25.73	25.74	22.9	1.67	1.68
30	15.92	15.96	15.38	-	0.91	0.91
32	23.89	23.99	24.22	-	1.39	1.39
33	170.79	170.36	170.36	-	-	-
34	20.62	20.95	20.97	-	1.96	1.98
35	-	51.34	51.38	-	-	3.65

a) Chemical shifts are expressed in ppm relative to internal TMS ($\nu_{TMS-^{13}C}$ = 25.1604 MHz) and are estimated to be accurate to within ± 0.04 ppm (δ_C) and ± 0.05-0.1 ppm (δ_H). ^{1}H chemical shifts are determined from selective proton decoupling experiments (see text). Solutions are 0.10 m in $CDCl_3$.

b) 3-Epi-fusidic acid methyl ester, 0.07 m in $CDCl_3$.

c) Cholestan-3β-ol; data from ref. (8) after conversion to the TMS scale using δ_{CS_2} = 192.8 ppm (see also ref. (10a) p. 440). The assignment of C12 and C16 has been reversed according to W. B. Smith, D. L. Deavenport, J. A. Swanzy, and G. A. Pate, J. Magn. Resonance 12, 15 (1973).

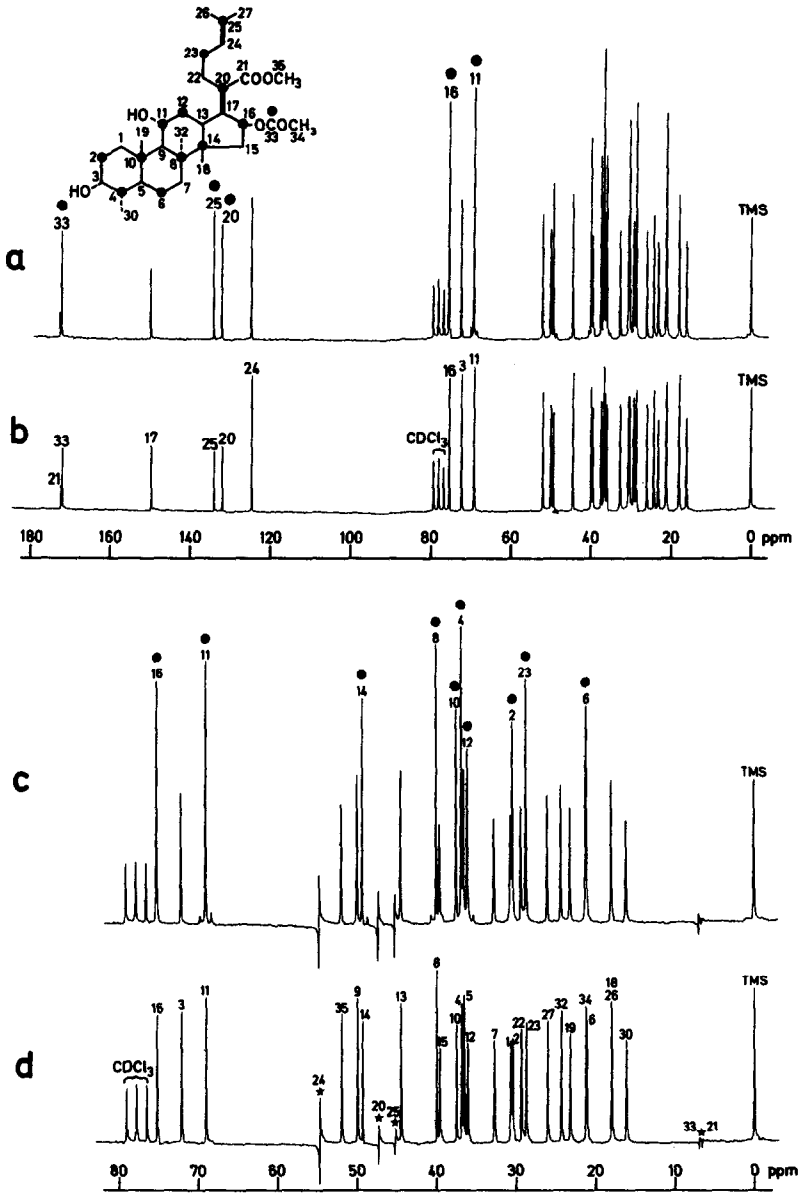

Figure 1. 25.16 MHz proton noise decoupled ^{13}C FT NMR spectra of (II) (b and d) and biosynthetically ^{13}C enriched ($CH_3{}^{13}COONa$) (II) (a and c); all 30.000 transients; 0.10 m in $CDCl_3$. Spectra a and b: 5000 Hz spectral width (SW), 0.8 sec acquisition time (AT), 1.0 sec pulse delay (PD). Spectra c and d: 2500 Hz SW, 1.6 sec AT, 0 sec PD. Enriched sites are indicated by (•) in the structural formula and in a and c. Signal folds in c and d are indicated by an asterisk (*). ^{13}C satellites due to one-bond ^{13}C-^{13}C couplings are observed near the signals for C11, C12, C8, and C14 ($^1J_{C11-C12}$ = 36 Hz) in c.

spectrum of (I); "cross-overs" of signals occur only for C6 and C34, as confirmed by selective decoupling. The ^{13}C and ^{1}H chemical shift data are summarized in Table 1.

As one of the obvious applications of the ^{13}C chemical shift assignment for (I) and (II), we have studied the biosynthetic incorporation of $CH_3{}^{13}COONa$ into fusidic acid. Although only ca. 1% incorporation of ^{13}C was observed for a sample of (I), isolated after growing the fungus Fusidium coccineum Fuckel-K. Tubaki in a suitable medium containing 0.6% $CH_3{}^{13}COONa$ (5), the ^{13}C spectrum (corresponding methyl ester, Figure 1) immediately permitted identification of the carbon positions where enrichment takes place. These are indicated in the structural formula of Figure 1 and fully confirm the proposed biosynthetic pathways (condensation followed by ring closure of two squalene units) to fusidic acid (5).

A detailed comparison of the ^{13}C chemical shifts obtained for the fusidane ring system with those reported for the cholestane steroids (8) is not warranted for steric reasons. It would nevertheless be of interest to see the extent to which the shifts are influenced by these factors. Thus we have included (Table 1) our data for 3-epi-fusidic acid methyl ester (III) for comparison with those of cholestan-3β-ol (IV) (8,10a). Even though large shift differences are observed for related carbons (e.g. C19) the relative orders of the chemical shifts for the ring A carbons (C1-C5,C10) and ring B carbons (C5-C10) are consistent.

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