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FIVE INGOL ESTERS AND A 17-HYDROXYINGENOL ESTER FROM THE LATEX OF EUPHORBIA KAMERUNICA. ASSIGNMENT OF ESTERS USING ^{13}C N.M.R. METHODS.

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Summary The structures of five ingol esters and a 17-hydroxyingenol ester from the latex of Euphorbia kamerunica have been determined. The ^{13}C n.m.r. spectra of these compounds have been assigned using 2D δ_C/δ_H correlations. The specific positions of attachment of esters have been assigned unambiguously using ^{13}C n.m.r. methods including 2D long range δ_C/δ_H correlations.

The diterpenoids of the Euphorbiaceae have been a source of great interest to organic chemists because of their biological activity.[1] These compounds often occur as mixed esters of the same nucleus and it is difficult to obtain pure compounds and to decide which ester is attached to the various positions of the diterpenoid nucleus. In the past partial hydrolysis has been used to solve the ester problem. However, while it has been reported[2] that intramolecular transesterification can occur during partial hydrolysis this has not always been taken into account[3] in the structural assignments. We have been able to circumvent the difficulties of transesterification using ^{13}C n.m.r. methods. Surprisingly there are few reports of ^{13}C chemical shift data for these compounds in the literature.[1] We now report the structural elucidation and the ^{13}C chemical shifts of five ingol esters (1), (2), (7), (10), and (16) [as its 3-deacetyl derivative (15)] and a 17-hydroxyingenol ester (17) from the latex of Euphorbia kamerunica.

Multiple preparative t.l.c. of the ether extract of the latex afforded several bands. The third band in order of increasing polarity gave a crystalline mixture of compounds (1) and (2) in the ratio 3:2. It was evident from the ^{1}H and ^{13}C n.m.r. spectra (Tables 1 and 2) by comparison with ingol tetra-acetate (3), whose relative stereochemistry has recently been reassigned,[4] that both compounds contain an ingol nucleus with two acetates and a methoxyl group. In addition there are resonances for a tiglate in (1) and a benzoate in (2). The chemical shift of H-8 indicated that the methoxyl group is attached to C-8 in both (1) and (2). Partial hydrolysis of the mixture yielded the corresponding 3-deacetyl derivatives (4) and (5) which were separated by preparative t.l.c. In both cases C-2 and C-3 exhibited the expected ^{13}C shifts, relative to (1) and (2), for removal of the 3-acetate. Further partial hydrolysis of (4) gave 8-O-methyl-ingol-12-acetate (6). Thus the tiglate in (1) and (4) must be attached to C-7. The benzoate group of (5), and hence of (2) was shown to be attached to C-7 by selective decoupling experiments in the fully coupled ^{13}C n.m.r. spectrum. Irradiation of H-7 (δ 5.59) resulted in removal of a coupling (^{3}J = 3 Hz) to the benzoyl carbonyl group which then appeared as a triplet, ^{3}J (H-ortho) = 3 Hz. Similarly for the acetate carbonyl carbon irradiation of H-12 (δ 4.87) removed a 4 Hz coupling (^{3}J) and left a quartet (^{2}J = 7 Hz). Thus the structures of (1) and (2) are established as 8-O-methyl-ingol-3,12-diacetate-7-tiglate and 8-O-methyl-ingol-3,12-diacetate-7-benzoate respectively.

The third ingol ester, less polar than (1) and (2), was assigned structure (7), ingol-

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3,8,12-triacetate-7-tiglate, on the basis of its spectroscopic properties (Tables 1 and 2). The presence of a 7-tiglate was established by 2D δ_C/δ_H correlation using polarisation transfer based on long range couplings, in particular the three bond coupling between H-7 and the tiglate carbonyl group. A more detailed description of the use of the above methods for determining sites of specific ester attachment will appear elsewhere.[5] Partial hydrolysis of (7) yielded inter alia ingol-12-acetate-8-tiglate (8)[6] which, on acetylation, afforded (9), isomeric with the natural ester (7). This result confirms the report[2] that ester transfer between the C-7 and C-8 hydroxyl groups can occur during partial hydrolysis and illustrates the hazards of assigning structures based on such evidence. Thus it seems likely that the compounds from E. kamerunica recently described[3] as the 8-tiglate (9), 8-angelate and 8-benzoate of ingol-3,7,12-triacetate are, in fact, the corresponding 7-esters, including (7). Comparison of the published data[3] for these compounds with our data supports this suggestion. The published data for a compound described[7] as ingol-3,7,8-triacetate-12-tiglate also bear remarkable similarity to those of (7). No evidence was presented for the location of the tiglate at C-12.

The least polar compound, ingol-3,8,12-triacetate-7-angelate (10), has spectroscopic properties which are virtually identical, apart from the angelate resonances, with those of the tiglate (7). The presence of a 7-angelate in (10) was proved by partial hydrolysis in two stages. First the 3-deacetyl derivative (11) was obtained. Further partial hydrolysis of (11) enabled the separation of the fifth ingol ester, which accompanied (10) as a minor contaminant, as its 3-deacetyl derivative (see below) and also afforded the 12-acetate-7-angelate (12), the 12-acetate-8-angelate (13) and the 12-acetate (14). Acetylation of (12) gave the natural product (10).

The 3-deacetyl derivative of the fifth ingol ester was readily assigned structure (15), 8-O-methyl-ingol-12-acetate-7-angelate, on the basis of its spectroscopic properties. Application of the 2D long range δ_C/δ_H correlation method described above confirmed the presence of the 7-angelate. Thus the natural ingol ester is 8-O-methyl-ingol-3,12-diacetate-7-angelate (16).

The ^{13}C chemical shifts of these ingol esters were largely assigned using 2D δ_C/δ_H correlations. The problem of the assignment of the epoxide carbons, C-4 and C-15, was solved by considering their long range proton couplings. In the coupled spectrum of (7) the signal at δ_C 73.4 is a sharp quartet (J 7 Hz) and that at δ_C 71.1 a broad doublet of triplets (J 2, 7 Hz). The 2D long range δ_C/δ_H correlations show that the δ 73.4 resonance is coupled to H-5, H-2 and H-1β whereas the δ 71.1 signal is coupled to H-2, H-1β and more weakly to H-1α. Using the crystal structure data for ingol tetra-acetate the torsion angles C-4, C-15, C-1, H-1α and C-15, C-4, C-5, H-5 are inferred to be -105.2° and 111.6° respectively, for which $^3J_{CH}$ values near zero are expected. Hence δ 73.4 is assigned to C-4 and δ 71.1 to C-15.

The most polar compound (17), m.p. 138-9°, [δ_H 4.98 (H-3), 5.37 (H-5), 4.07 and 4.16 (ABq, J 11.7 Hz, 2H-17), 4.11 and 4.55 (ABq, J 12.2 Hz, 2H-20), 4.32 (H-8), 0.89 (H-13), 1.11 (H-14)], has three acetates and an angelate attached to an ingenol nucleus bearing an acyloxymethyl group on C-15. The ^{13}C resonances of the geminal methyls in the ingol and ingenol series appear at approximately 16 ppm for the β-methyl and 29 ppm for the α-methyl. Introduction of an acyloxy function to either methyl would be expected to cause an upfield shift of the remaining methyl. Since the only t-methyl in (17) resonates at 24.1 ppm it must be α, i.e. C-16, and hence the acyloxymethyl is C-17 (Table 3).

The position of attachment of the angelate was determined by partial hydrolysis which afforded the 17-acetate-3-angelate (18) [δ_H 4.13 (s, 2H-20), 4.04 (s, H-5)], the 17-acetate-20-angelate (19) [δ_H 4.39 (s, H-3), 3.65 (s, H-5)] and the 20-angelate (20) [δ_H 4.41 (s, H-3), 3.69 (s, H-5), 3.74 and 3.84 (ABq, J 11.7 Hz, 2H-17)]. Formation of (20) excludes the possibility of a 17-angelate in (17) since ester transfer between C-17 and C-20 is impossible. Acetylation of (18) yielded the natural compound whereas acetylation of (19) afforded the isomer (21) [δ 4.95 (H-3), 5.40 (H-5), 4.13 and 4.24 (2H-17), 4.31 and 4.55 (2H-20)]. It follows, therefore, that the natural ester (17), the most irritant of those isolated, is 17-acetoxyingenol-5,20-diacetate-3-angelate. The ^{13}C chemical shifts of the carbonyl group of the ester attached to C-3 are at lower field than normal, presumably as a result of H-bonding to the tertiary hydroxyl group. Thus the ^{13}C shift of the angelate carbonyl is 167.3 ppm in (21) and moves downfield to 168.8 ppm in the natural ester (17). Similarly one acetate carbonyl is more deshielded than usual in (21) and resonates at 172.4 ppm. The formation of the 20-angelate derivatives (19) and (20) shows that, as in the ingol series, ester transfer can occur during partial hydrolysis.

Complete hydrolysis of (18), followed by acetylation, afforded 17-hydroxyingenol tetra-acetate (22) whose ^1H n.m.r. spectrum [6.06 (H-1), 4.93 (H-3), 5.37 (H-5), 6.21 (H-7), 4.37 (H-8), 0.93 (H-13), 1.15 (H-14), 4.22 and 4.11 (ABq, J 11.9 Hz, 2H-17), 4.57 and 4.14 (ABq, J 12.7 Hz, 2H-20)] bears a striking similarity to that published[8] for a compound described as 16-hydroxyingenol tetra-acetate. The ^1H spectra of the isomers were predicted[8] to differ and we suspect that the literature compound[8] is identical to (22).

Table 1. ^{13}C Chemical Shifts of Ingol Derivatives ($CDCl_3$)

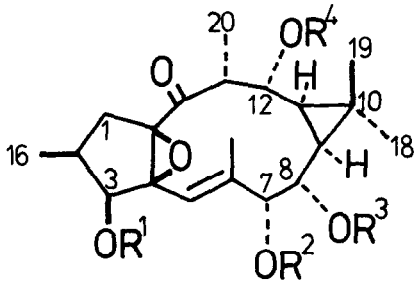
	(3)	(10)	(15)	(7)	(9)	(1)	(2)	(4)	(5)
1	31.3	31.5	31.7	31.5	31.4	31.5	31.5	31.8	31.7
2	29.3	29.5	31.9	29.5	29.3	29.4	29.4	31.9	31.9
3	76.2	76.5	76.1	76.6	76.3	76.8	76.9	76.1	75.9
4	73.3	73.4	75.7	73.4	73.4	73.5	74.4	75.7	75.7
5	117.0	117.0	117.5	116.8	116.8	116.7	117.1	117.3	117.6
6	139.5	139.8	139.5	139.9	139.9	140.0	139.7	139.5	139.2
7	76.8	76.6	73.6	76.7	77.0	74.0	74.6	73.7	74.3
8	71.2	71.5	78.8	71.7	71.2	78.8	78.8	78.9	78.9
9	24.7	25.1	27.2	25.0	25.0	27.2	27.2	27.1	27.1
10	19.3	19.3	19.2	19.3	19.3	19.3	19.4	19.2	19.2
11	30.6	30.7	30.5	30.7	30.8	30.5	30.5	30.4	30.5
12	70.6	70.6	71.0	70.6	70.8	71.0	71.0	70.8	71.0
13	42.9	43.1	42.9	43.1	43.0	43.0	43.1	43.0	43.0
14	207.4	207.6	207.8	207.6	207.6	207.6	207.6	207.8	207.8
15	71.0	71.1	72.8	71.1	71.1	71.1	71.2	72.8	72.8
16	16.9	16.9	16.1	16.9	17.0	16.9	16.9	16.1	16.1
17	17.3	17.5	17.8	17.5	17.4	17.7	17.8	17.8	17.8
18	29.0	29.1	29.2	29.2	29.2	29.3	29.3	29.3	29.3
19	16.0	16.1	16.5	16.1	16.1	16.5	16.5	16.5	16.5
20	13.3	13.4	13.2	13.3	13.4	13.3	13.3	13.2	13.2

Table 2.[9] ^1H Chemical Shifts of Ingol Derivatives

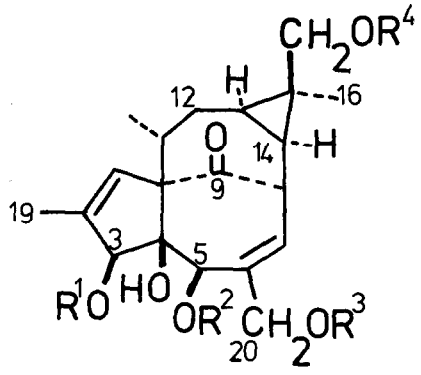
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
3	5.20	5.13	5.29	4.24	4.19	4.37	5.22	4.31	5.35	5.23	4.25	4.25	4.33	4.30	4.25
7	5.33	5.54	5.03	5.42	5.59	4.34	5.21	4.26	5.12	5.20	5.29	5.23	4.28	4.13	5.47
8	2.91	2.99	4.52	2.90	2.98	2.85	4.58	4.55	4.64	4.58	4.59	3.55	4.62	3.36	2.90
12	4.85	4.90	4.81	4.85	4.87	4.85	4.87	4.86	4.88	4.86	4.86	4.87	4.87	4.82	4.85

Table 3. ^{13}C Chemical Shifts of Ingenol Derivatives (17) and (22)

	(17)	(22)		(17)	(22)		(17)	(22)		(17)	(22)
1	131.5	131.8	6	135.9	135.8	11	38.5	38.6	16	24.1	24.2
2	133.7	133.6	7	130.8	131.0	12	30.6	30.8	17	65.6	65.7
3	81.5	82.0	8	43.1	43.1	13	23.1	23.2	18	16.5	16.6
4	85.7	85.7	9	204.6	204.6	14	23.9	24.0	19	15.3	15.4
5	74.6	74.6	10	71.9	71.8	15	27.5	27.7	20	65.6	65.7



	R^1	R^2	R^3	R^4
(1)	Ac	tig	Me	Ac
(2)	Ac	PhCO	Me	Ac
(3)	Ac	Ac	Ac	Ac
(4)	H	tig	Me	Ac
(5)	H	PhCO	Me	Ac
(6)	H	H	Me	Ac
(7)	Ac	tig	Ac	Ac
(8)	H	H	tig	Ac
(9)	Ac	Ac	tig	Ac
(10)	Ac	ang	Ac	Ac
(11)	H	ang	Ac	Ac
(12)	H	ang	H	Ac
(13)	H	H	ang	Ac
(14)	H	H	H	Ac
(15)	H	ang	Me	Ac
(16)	Ac	ang	Me	Ac



	R^1	R^2	R^3	R^4
(17)	ang	Ac	Ac	Ac
(18)	ang	H	H	Ac
(19)	H	H	ang	Ac
(20)	H	H	ang	H
(21)	Ac	Ac	ang	Ac
(22)	Ac	Ac	Ac	Ac

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9. Only chemical shift data are given. Multiplicities and coupling constants are essentially as reported for the parent compounds.

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