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THE STRUCTURE OF ENUKOKURIN, A NEW JATROPHANE DITERPENOID FROM THE LATEX OF *EUPHORBIA LATERIFLORA*

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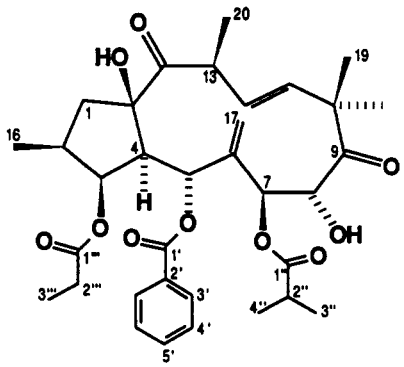
ABSTRACT.—The structure of enukokurin [**1**], a novel jatrophane ester from the latex of *Euphorbia lateriflora*, has been assigned on the basis of its ^1H and ^{13}C nmr spectroscopic properties. The positions of its ester linkages were determined using 2D long-range δ_C/δ_H correlation experiments and its relative stereochemistry by nOe difference spectroscopy.

One of the members of the Euphorbiaceae family found in southern regions of Nigeria is *Euphorbia lateriflora* Schum and Thonner whose local name is "Enukokure." Its latex is used as a cure for ringworm and, in dilute aqueous solution, as a purgative. Our interest in the chemistry of this plant arose from the traditional medicinal uses of its latex. Members of the Euphorbiaceae family are well known for producing a range of diterpenoid esters (1). Some of these are highly irritant and have tumor-promoting activity while others have cytotoxic activity. No previous work has been reported on the latex of *E. lateriflora*. We now report the isolation, characterization, and structural elucidation of enukokurin [**1**], a new jatrophane diterpenoid ester.

RESULTS AND DISCUSSION

The latex of *E. lateriflora* was extracted in the cold with EtOH and the solid obtained partitioned into petroleum-ether-soluble and Et_2O-soluble components. The Et_2O-soluble extract exhibited slight irritant properties. Multiple preparative tlc of the Et_2O extract afforded several bands, the least polar of which gave enukokurin [**1**] as a crystalline compound.

Enukokurin [**1**], $C_{34}H_{44}O_{10}$, has ^1H and ^{13}C nmr properties consistent with the presence of two secondary and two tertiary methyl groups, two ketones, a *trans* disubstituted double bond, an exomethylene, two hydroxyl groups (one secondary and one tertiary), and three secondary esters, a propanoate, a benzoate and a 2-methylpropanoate. Thus it is a derivative of a bicyclic diterpenoid: probably, in the absence of cyclopropane resonances, a jatrophane (1). By analogy with kansuinine B [**2**] (2) and esulone A [**3**] (3), the chemical shift and coupling constant data are consistent with **1** as



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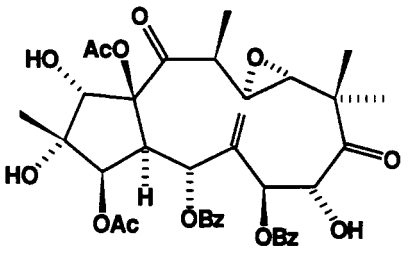

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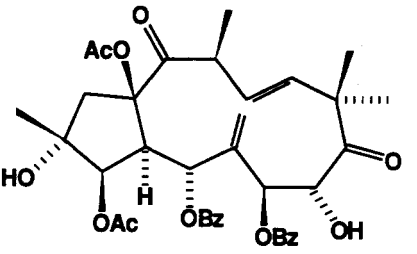

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a plausible working structure for enukokurin. The ^{1}H-nmr spectrum of enukokurin reveals H-7 as a singlet and H-8 as a doublet ($J = 9.6$ Hz) coupling with the secondary hydroxyl proton. These features are also present in the spectra of kansuinine B and esulone A. The protons attached to C-1 and C-2 of enukokurin form a strongly coupled system.

The assignment of the protonated ^{13}C resonances was achieved by a 2D direct δ_C/δ_H correlation experiment. Evidence for the position of attachment of the various esters was readily obtained from a 2D long-range δ_C/δ_H correlation experiment (4) which was also used to elucidate structure **1** independently. The defocusing (Δ_1) and refocusing (Δ_2) delays, which were not optimized, were 45 ms and 20 ms, respectively. The three ester carbonyls are distinguishable by their chemical shifts. The carbonyl at δ_C 173.4 correlates with the propanoate signals 2H-2‴ and 3H-3‴ and also with H-3, indicating that it is attached to the oxygen at C-3. Similarly the carbonyl at δ_C 164.7 correlates with the *ortho* protons of the benzoate and also with H-5 while the carbonyl at δ_C 176.3 correlates with the two methyl groups 3H-3″ and 3H-4″ of the 2-methylpropanoate and also with H-7. Thus the potentially difficult task of assigning the ester attachments was completed unambiguously and in a simple manner.

In order to use the 2D long-range δ_C/δ_H correlation results for structural elucidation we assume that, with our choice of delays [optimal for $J(^{13}C^1H) = 11$ Hz], the long-range $^{13}C^1H$ correlations observed arise from either 2- or 3-bond couplings and the arguments are presented in such a manner that the distinction of the two possibilities emerges as a necessary consequence of the arguments. For example, coupling of a quaternary carbon to 2° methyl protons cannot occur via a two-bond coupling. Also we only use the presence and not the absence of correlations in our arguments in order to overcome the problem (5) that one-bond coupling causes intensity modulation of long-range correlations when the original Freeman-Morris pulse sequence (6) is used. Just before this work was carried out, sequences using BIRD and TANGO pulses were being developed (7) to remove this effect and have been extensively developed subsequently (8), but at the time we found the conventional sequence adequate for our purposes. In the presentation of the structural elucidation which follows, the numbering of structure **1** is used but the numbers serve purely as arbitrary labels until the structure is established.

Starting with the ketone at δ_C 212.1 (C-14) we observe correlation with the 2° methyl group 3H-20. This in turn shows correlation with one end, C-12, of the disubstituted double bond. The other end, C-11, shows correlation with two 3° methyl groups which, as neither is a substituent of the double bond, must be part of a *gem*-dimethyl fragment. The protons of these two methyl groups attached to C-10 also show correlation to the second ketone (C-9) at δ_C 210.7. C-9 shows no other strong correlations and progress is temporarily blocked. However, C-7 shows correlations to both exomethylene protons, and a correlation to H-5 bridges the exomethylene group. C-5

also shows correlations to both exomethylene protons, the correlation at δ_H 6.02 being strong compared to the weaker one at δ_H 5.75. This is the reverse of the situation for C-7 and enables H-17(*Z*) to be assigned to δ_H 6.02 and H-17(*E*) to δ_H 5.75. This conclusion is valid even in the presence of intensity modulation by one-bond coupling, as the relative order of the size of the correlations will be unchanged as long as both long-range couplings are smaller than the coupling giving the maxiumum response with the delays used. H-5 shows a large splitting (10.5 Hz) that must be a vicinal coupling to H-4, and this proton shows a correlation to our starting point C-14; however, the 3° hydroxyl group also shows a correlation to C-14; therefore the quaternary carbon-bearing oxygen must be placed as C-15 between C-4 and C-14. C-14 also correlates with a pair of protons, which therefore must form part of a C-1 methylene group attached to C-15. C-1 correlates with a 2° methyl group 3H-16, whose associated methine carbon C-2 correlates with H-1β. 3H-16 also correlates with the ester-bearing carbon C-3, and H-3 correlates with C-15, thus completing formation of a five-membered ring. Apart from the esters attached to C-3, C-5, and C-7, the only other structural components left at this point are a 2° hydroxyl moiety and a free bond at both C-7 and C-9. The only solution is to form a twelve-membered ring by inserting C-8 between C-7 and C-9. This completes the basic skeletal framework of enukokurin.

The relative stereochemistry of enukokurin as in **1** can be deduced from consideration of the results of nOe difference experiments. A convenient point of reference is H-4, which is assumed to be α. Irradiation of H-4 produces a strong nOe at H-13 (11%). Models indicate that such an effect is possible only with a *trans* ring junction and with an α configuration of H-13. Irradiation of H-13 gives a significant nOe at H-11 (8%) in addition to the expected effects at H-4 (9%) and 3H-20 (3%). The resonance for H-12 has a 6% nOe on irradiation of 3H-20. These results indicate clearly the orientation of the double bond with H-12 projecting above the plane of the molecule and permit a distinction to be made between the resonances of the geminal methyl groups. Irradiation of the methyl signal at δ_H 1.14 causes an nOe of H-12 (15%) with a much smaller effect at H-11 (2%). This resonance is therefore 3H-19. Irradiation of the other tertiary methyl resonance (3H-18) at δ_H 1.25 gives the expected large nOe at H-11 (8%) with a smaller effect at H-12 (3%).

The stereochemistry along the lower half of the medium ring may be argued as follows. The large coupling constant (J = 10.5 Hz) between H-4 and H-5, taken in conjunction with the observation of nOe's from 15-OH of both H-5 (3.8%) and H-17(*E*) (2.4%) requires that H-5 is β. The combination of nOe's from H-4 to H-7 (19%), 3H-19 to H-8 (7%) and H-8 to H-17(*Z*) (5%) and the zero ^3J(H-7,H-8) is best accommodated by having H-7α and H-8β. With H-7β these conditions can be more or less satisfied apart from the nOe between H-8 and H-17(*Z*). These results indicate that the exomethylene group projects above the plane of the molecule. It should also be noted that they confirm the assignments of the exomethylene protons arrived at on the basis of the 2D long-range δ_C/δ_H correlations.

The overlap of H-2 and H-1α in the ^{1}H nmr spectrum of enukokurin is unfortunate as it nullifies the obvious approach to the determination of the configuration at C-2, i.e., in the use of nOe of H-2. The difficulty is readily overcome by using C_6D_6 as solvent. This results in differential shifts of the various protons. The multiplet arising from H-2 is clearly visible, separate from the 2H-1 resonances but partially overlapping with H-2″, the 2-methylpropanoate methine. Irradiation of H-4 results in an nOe (3%) of the H-2 multiplet in addition to the expected nOe's of H-7 (19%), H-3 (13%), and H-13 (10%).

The reverse experiment, irradiation of H-2, is complicated by the fact that the H-2 and H-2″ multiplets overlap. However differential partial saturation was achieved in

two multiple selective irradiation experiments (9,10); this was apparent because the enhancements of three signals increased fourfold on going from the experiment intended to saturate H-2 to the experiment intended to saturate H-2″, whereas the enhancements of four signals were essentially unchanged. On the basis that all enhancements caused by H-2″ must increase fourfold, it is possible to infer that the unchanged enhancements are caused by H-2. It can also be deduced that the approximate level of saturation in the first experiment is 37% of H-2 and 12% of H-2″, and in the second experiment 38% of H-2 and 48% of H-2″. Scaling up to 100% saturation produces the following results: H-2 gives nOe's at H-3 (8%), H-4 (2%), H-13 (5%), and 3H-16 (1%), while H-2″ gives nOe's of the methyl resonances 3H-3″ and 3H-4″ of the 2 methylpropanoate (both 2%) and, unexpectedly but explicably, of the *ortho*-protons of the benzoate ester (2%). These results are consistent with the presence of α hydrogens at C-2 and C-3.

It is not surprising that the relative stereochemistry deduced for enukokurin [**1**] is the same as that of esulone A [**3**] (3) and kansuinine B [**2**] (2), as the coupling data of the three molecules are virtually identical. The conformation of enukokurin which best accommodates the nOe evidence is very similar to the conformation of esulone A derived from X-ray analysis (3). The drawing in Manners and Wong (3) that shows kansuinine B with a 7α-oxygen substituent is erroneous and probably results from the use of re-entrant angles in the diagrams of the original paper (2). No direct evidence is presented for the absolute configuration of enukokurin but because its small positive $[\alpha]_D$ is similar to those of esulone A and kansuinine B we have assumed that all three compounds share the same absolute configuration.

Enukokurin is an additional member of the small group of jatrophane diterpenoids from the Euphorbiaceae. In addition to the compounds discussed by Uemura *et al.* (2) and Manners and Wong (3) or reviewed by Evans and Taylor (1), several jatrophanes have been isolated from *Euphorbia helioscopia* (11) and *Euphorbia characias* (12).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—Melting points were determined on a Kofler hot stage microscope and are uncorrected. Specific rotations were obtained on an AA-100 automatic digital polarimeter. Nmr spectra were run at 4.7 T at ambient probe temperature (ca. 24°) of $CDCl_3$ solutions and chemical shifts reported with respect to $CHCl_3$ at δ_H 7.25 and $CDCl_3$ at δ_C 77.0. Digital resolution in the 2D δ_C/δ_H correlation experiments was 5 Hz/data point for the ^{13}C axis and 6 Hz/data point for the ^1H axis. Mass spectra were determined using MS 12 (low resolution) and MS 902S (high resolution) instruments. Preparative tlc utilized Si gel GF_{254} coated to 0.5 mm thickness and viewed under uv lamp. Elution of plates was done with CH_2Cl_2/MeOH mixtures.

PLANT MATERIAL.—The latex of *E. lateriflora* was collected from plants growing on the campus of Obafemi Awolowo University, Nigeria, and authenticated by Mr. Jaiyeola, Department of Botany, O.A.U., Ile-Ife. A voucher specimen was deposited in the Herbarium of the department.

EXTRACTION OF THE LATEX OF *E. LATERIFLORA*.—Latex (250 ml) was tapped over a period of 4 months. It was each time taken up in EtOH and kept frozen. Subsequent extraction with cold EtOH (2.5 liters) afforded a solution which was concentrated under vacuum at 35–40°. The residue was taken up in MeOH-H_2O (9:1) and then extracted with petroleum ether. The petroleum ether extract was set aside, and the mother liquor was concentrated under vacuum and later extracted with Et_2O. The Et_2O extract was washed with H_2O, dried over $MgSO_4$, and finally evaporated to a gum (28 g) which contained the irritant components of the latex.

ISOLATION OF ENUKOKURIN [**1**].—A sample (2 g) of the Et_2O extract was subjected to preparative chromatography on 16 (20 cm × 20 cm) plates, eluting twice with CH_2Cl_2-MeOH (50:1). The least polar band (900 mg) contained enukokurin. Crude enukokurin (500 mg) was further subjected to preparative tlc using 10 plates, eluting four times with CH_2Cl_2-MeOH (100:1). The second band of this gave pure enukokurin [**1**] as needles, mp 193–195° (Et_2O); $[\alpha]^{25}D$ +30.8 (c = 12.40, $CHCl_3$); ir ν max (CCl_4) 3530, 3465, 1741, 1729, 1713, 1703 cm^{-1}; found m/z 612.2934, $C_{34}H_{44}O_{10}$ requires 612.2922; ^1H nmr δ_H 1.85 (m, H-1β), 2.3 (m, H-1α, H-2), 5.70 (t, J = 3.9 Hz, H-3), 2.94 (dd, J = 10.5, 4.1 Hz, H-4), 5.87 (d, J = 10.5 Hz, H-5), 5.64 (s, H-7), 4.58 (d, J = 9.6 Hz, H-8), 3.10 (d, J = 9.6 Hz, exchange-

able with D_2O, 8-OH), 5.96 (d, J = 16.1 Hz, H-11), 5.67 (dd, J = 16.1, 9.6 Hz, H-12), 3.57 (dq, J = 9.6, 6.5 Hz, H-13), 4.17 (s, exchangeable with D_2O, 15-OH), 0.98 (d, J = 6.3 Hz, 3H-16), 5.75 [s, H-17(*E*)], 6.02 [s, H-17(*Z*)], 1.14 (s, 3H-19), 1.25 (s, 3H-18), 1.38 (d, J = 6.5 Hz, 3H-20), 1.53 (septet, J = 6.9 Hz, H-2″), 0.52 and 0.78 (both d, J = 6.9 Hz, 3H-3″ and 3H-4″), 2.20 (q, J = 7.6 Hz, 2H-2‴), 0.85 (t, J = 7.6 Hz, 3H-3‴), 7.82 (m, 2H-3′, 2H-7′), 7.33 (m, 2H-4′, 2H-6′), 7.47 (m, H-5′); ^{13}C nmr δ_C 46.4 (t, C-1), 38.5 (d, C-2), 77.2 (d, C-3), 50.1 (d, C-4), 73.9 (d, C-5), 137.8 (s, C-6), 63.5 (d, C-7), 71.5 (d, C-8), 210.7 (s, C-9), 48.1 (s, C-10), 135.5 (d, C-11), 132.8 (d, C-12), 43.5 (d, C-13), 212.1 (s, C-14), 84.2 (s, C-15), 13.8 (q, C-16), 125.4 (t, C-17), 25.3 (q, C-19), 23.3 (q, C-18), 20.9 (q, C-20); benzoate 164.7 (C-1′), 129.6 (C-2′), 129.5 (C-3′, C-7′), 128.2 (C-4′, C-6′), 132.9 (C-5′); 2-methylpropanoate 176.3 (C-1″), 33.2 (C-2″), 17.5 and 18.9 (C-3″, C-4″); propanoate 173.4 (C-1‴), 27.2 (C-2‴), 8.9 (C-3‴).

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