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MACROCYCLIC DITERPENE ESTERS FROM *EUPHORBIA ROYLEANA**

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Key Word Index—*Euphorbia royleana*, Euphorbiaceae, ingol-esters, macrocyclic diterpenes

Abstract—An ether soluble resin was prepared by extraction and partition of the latex of *Euphorbia royleana* This resin demonstrated pronounced pro-inflammatory activity on mammalian skin and was separated into biologically active and inactive fractions by dry column chromatography The inactive fraction was further separated by adsorption and partition thin-layer methods The least polar zone consisted of an inseparable mixture of four tetra-esters of the macrocyclic diterpene ingol, whilst the more polar fraction consisted of a mixture of four tri-esters of the same diterpene

INTRODUCTION

Macrocyclic diterpenes of the jatrophane [1] and lathyrane [2] classes have previously been isolated from both herbaceous and succulent varieties of the genus *Euphorbia* [3] The ingol-esters are compounds of the lathyrane type and comprise a small group of new natural products only isolated to date from four succulent *Euphorbias* [3] Species of this genus commonly produce the tetra-cyclic diterpene ingenol [4], however, the macrocyclic derivatives may be implicated in the biosynthesis of this compound [5] Ingol-esters do not exhibit the toxic pro-inflammatory actions of ingenol-esters on mammalian skin [3] but certain *Euphorbia* macrocyclic derivatives have demonstrated cytotoxic actions *in vitro* [6, 7] *Euphorbia royleana* L is a large succulent species naturalized to gardens in parts of Egypt as an ornamental variety In this communication we describe the separation of ingol-esters from the toxic ingenol analogues and the structural elucidation of eight esters of ingol

RESULTS AND DISCUSSION

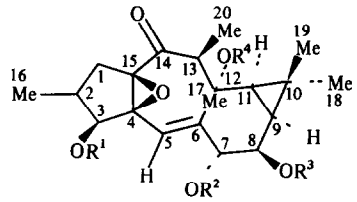
A caustic resin was produced from the latex of *E royleana* by partition and extraction methods This resin was separated by dry column chromatography, monitored by an *in vivo* pro-inflammatory assay [8], into several fractions Two of these fractions, although inactive in the biological test system used, contained esters of ingol (**3–10**) Further purification of the first of these fractions by means of adsorption and partition preparative TLC techniques produced mixture A Attempts at further separation resulted in the production of decomposition products and serious loss of material on recovery Esters of ingol (**1**) separate chromatographically according to the number of free hydroxyl groups on the nucleus, and mixtures of tetra-esters appear as a single fraction by conventional techniques [9] The composition of such inseparable mixtures has previously been obtained by spectroscopic methods [9–11]

Complete alkaline hydrolysis of A followed by acetylation produced ingol-tetra-acetate, recognized by comparison of its ^{1}H NMR, MS and TLC characteristics to an authentic sample The absolute configuration of ingol-tetra-acetate was recently obtained by X-ray and ^{1}H NMR methods [12] Mixture A, from observations of its mass and ^{1}H NMR spectra, consisted of four tetra-esters of ingol (**3–6**) in which three hydroxyl groups were acetylated whilst the third exhibited either an angelate (**3**), benzoate (**4**), tigliate (**5**) or α-methylbutyrate (**6**) moiety From the intensity of M^+ ions in the MS of mixture A and from integrals of its ^{1}H NMR spectrum this mixture consisted approximately of 27% angelate, 21% benzoate, 27% tigliate and 25% α-methylbutyrate esters of ingol-triacetate (**3–6**)

Fraction two from the column was similarly separated by adsorption and partition TLC to produce an inseparable mixture B The ^{1}H NMR and mass spectra of mixture B showed that it consisted of a mixture of four tri-esters of ingol (**7–10**) in which two hydroxyl groups were acetylated whilst a fourth exhibited one of either angelate (**7**), benzoate (**8**), tigliate (**9**) and α-methylbutyrate (**10**) in similar proportions to mixture A (see above)

Extensive decoupling experiments of the ^{1}H NMR spectra of ingol-esters together with degradative and synthetic methods [3] have previously confirmed that ingol-tetra-esters have ester groups at 3, 7, 8 and 12 of the nucleus The upfield shift in the signal for the proton at C-7 from δ5 12 in A to δ4 14 in mixture B confirmed that three acyl residues were present at 3, 8 and 12 in B and further suggested that in mixture A an extra acetate group was present at position 7 Mixture B was hydrolysed with alcoholic potassium hydroxide to produce the mono-ester ingol-12-acetate (**2**), identified by comparison of its ^{1}H NMR spectrum with an authentic sample Accordingly in mixtures A and B the higher MW acyl group could be present at either C-3 or C-8 of the ingol nucleus Paucity of material precluded further hydrolysis reactions under controlled conditions [9] However, in all ingol-esters isolated to date an acetate moiety was present at C-3 [3], and on biosynthetic grounds the longer chain acyl residues of mixtures A and B can tentatively be assigned to the C-8 position Angelic and tiglic acids are

*Part 11 in the series "Constituents of Egyptian Euphorbiaceae"



		R^1	R^2	R^3	R^4
Ingol	**1**	H	H	H	H
Ingol 12 - acetate	**2**	H	H	H	Acetate
Compounds of mixture A					
	3	Acetate	Acetate	Angelate	Acetate
	4	Acetate	Acetate	Benzoate	Acetate
	5	Acetate	Acetate	Tigliate	Acetate
	6	Acetate	Acetate	α - Methyl butyrate	Acetate
Compounds of mixture B					
	7	Acetate	H	Angelate	Acetate
	8	Acetate	H	Benzoate	Acetate
	9	Acetate	H	Tigliate	Acetate
	10	Acetate	H	α - Methyl butyrate	Acetate

cis/trans isomers and both were present in these mixtures because of the occurrence of the tigliate olefinic proton at δ6 85 and the angelate olefinic proton at δ6 09 in their ^{1}H NMR spectra [13] Fraction A therefore contains the new ingol-ester 8-α-methyl butyrol-ingol-3,7,12-triacetate (**6**) in admixture with three tetra-esters (**3–5**) previously obtained from *E kamerunica* [9] whilst B consisted of three new tri-esters of ingol (**7**, **8** and **10**) together with 8-tigloyl-ingol-3,12-diacetate (**9**) previously obtained from *E lactea* [14]

EXPERIMENTAL

The latex of *E royleana* was collected directly in MeOH, from plants growing in Orman Garden, Gizia, Egypt

Extraction MeOH preserved latex (250 g) was evaporated to dryness below 45° The residue was exhaustively extracted with Me_2CO at room temp for one week After evaporation of the Me_2CO the residue was dissolved in MeOH–H_2O (3 1) and lipids and steroids were removed by partition with hexane The aq phase was re-extracted with Et_2O and the Et_2O-soluble resin was tested for pro-inflammatory activity using a mouse-ear assay [8] Resin (1 g) was separated by dry CC on 70 g of deactivated silica gel using toluene–Me_2CO (19 1) as the developing solvent Fractions of 50 ml vol were collected and monitored by TLC and biological tests Two non-biologically active fractions were separately obtained on the basis of their R_f-values and colour reactions to H_2SO_4 after heating to visualize the plates

Fraction 1 was further purified by prep TLC on buffered silica gel H 500 μ layers using toluene–Me_2CO (9 1) solvent (R_f 0 43) This zone was finally purified by means of partition TLC using silica gel H 500 μ layers coated with 15% propylene glycol and heptane–Et_2O (9 1) as solvent, followed by adsorption TLC as before using $CHCl_3$–Et_2O–C_6H_6 (1 3 3) as solvent to give mixture A EIMS (170°, 70 eV) *m/z* 576 ($C_{31}H_{44}O_{10}$), 574 ($C_{31}H_{42}O_9$) and 596 ($C_{33}H_{40}O_{10}$), fragment ions were exhibited in this spectrum at 556, 537, 516, 475, 456, 454, 396, 394, 330, 312, 294, 105 and 83 (base peak), ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ7 42–8 02 (5H, arom), 6 85 (*m*, 1H, olefinic), 6 09 (*m*, 1H, olefinic), 5 59 (*s*, 1H), 5 34 (*d*, *J* = 8 7 Hz, 1H), 5 12 (*s*, 1H), 4 88 (*dd*, *J* = 3 7, 11 0 Hz, 1H), 2 95 (*m*, 1H), 2 85 (*m*, 1H), 2 52 (*m*, 1H), 2 18 (3H, acetyl), 2 13 (3H, acetyl), 2 10 (3H, acetyl), 2 08 (*s*, 3H), 1 81 (6H), 1 26 (*s*, 3H), 1 08 (*m*, 8H), 0 97 (*m*, 3H), 0 82 (*m*, 3H) Mixture A was hydrolysed with 0 5 M KOH in dry MeOH to produce a base line product by TLC which was identified after conversion to its acetate with Ac_2O–C_5H_5N (2 1) as ingol-tetra-acetate by comparison with an authentic sample (TLC, MS, ^{1}H NMR) [3]

Fraction 2 This fraction was further purified by means of adsorption TLC on silica gel as before using toluene–Me_2CO (9 1) as solvent (R_f 0 28) Final purification was achieved using partition TLC with heptane–Et_2O (9 1) as solvent followed by adsorption TLC with $CHCl_3$–Et_2O–C_6H_6 (1 3 3) as solvent Mixture B exhibited the following spectral data EIMS *m/z* 532 ($C_{29}H_{40}O_9$), 534 ($C_{29}H_{42}O_9$) and 554 ($C_{31}H_{38}O_9$) and fragment ions at 494, 474, 472, 434, 414, 412, 330, 312, 105 (base peak) and 83, ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ7 42–8 02 (5H, benzoate), 6 85 (*m*, olefinic 1H), 6 09 (*m*, olefinic, 1H), 5 60 (*s*, 1H), 5 22 (*d*, 1H), 4 4 (*s*, 1H), 4 91 (*s*, 1H), 4 7 (*dd*, 1H), 2 95 (*m*, 1H), 2 85 (*m*, 1H), 2 56 (*m*, 1H), 2 20 (3H, acetyl), 2 16 (3H, acetyl), 2 04 (*s*, 3H), 1 84 (*m*, 6H), 1 26 (*s*, 3H), 1 10 (*m*, 9H), 0 97 (*m*, 3H), 0 82 (*m*, 3H) Mixture B was hydrolysed as before to produce a base line product by means of TLC which was identified as ingol-12-acetate by comparison with an authentic sample (^{1}H NMR)

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REFERENCES

1 Uemura, D and Hirata, Y (1975) *Tetrahedron Letters* 1697
2 Adolf, W, Hecker, E, Balmain, A, Lohmme, M F, Nakatani, Y, Ourisson, G, Ponsinet, G, Pryce, R J, Santhanakrishnan, T S, Matyukhina, G and Saltikova, I A (1970) *Tetrahedron Letters* 2241
3 Evans, F J and Taylor, S E (1983) *Progress in the Chemistry of Organic Natural Products* (Herz, W, Grisebach, H and Kirby, G W, eds) Vol 44, p 1
4 Evans, F J and Kinghorn, D (1977) *Bot J Linn Soc* 74, 23
5 Ghisalberti, E L, Jefferies, P R, Payne, T G and Worth, G K (1973) *Tetrahedron Letters* 403
6 Sahai, R, Rastogi, R P, Jakupovic, J and Bohlmann, F (1981) *Phytochemistry* **20**, 1665
7 Abo, K and Evans, F J (1981) *Phytochemistry* **20**, 2535
8 Evans, F J and Schmidt, R J (1979) *Inflammation* **3**, 215
9 Abo, K and Evans, F J (1981) *Planta Med* **43**, 392
10 Opferkuch, H J and Hecker, E (1973) *Tetrahedron Letters* 3614
11 Seip, E M and Hecker, E (1983) *Phytochemistry* **22**, 1791
12 Lotter, H, Opferkuch, H J and Hecker, E (1979) *Tetrahedron Letters* 77
13 Schmidt, R J and Evans, F J (1977) *Lloydia* **40**, 225
14 Upadhyay, R R and Hecker, E (1975) *Phytochemistry* **14**, 2514

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VESTOLIDE, A GUAIANOLIDE FROM *VICOA VESTITA**

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Key Word Index—*Vicoa vestita*, Asteraceae, guaianolide, vestolide

Abstract—A new sesquiterpene lactone, vestolide, of the guaiane series was isolated from the aerial parts of *Vicoa vestita* Its structure was established on the basis of chemical and spectral evidence

INTRODUCTION

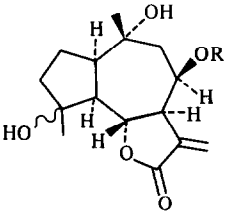
Vicoa vestita (Wall ex DC) Benth ex Hook f (syn *Inula vestita* Wall ex DC) is a woolly herb distributed all over India The alcoholic extract of the aerial parts of the plant was found to possess oxytocic activity when tested at this Institute The activity was later confirmed in its *n*-butanol-soluble fraction The chemical investigation of the *n*-butanol fraction carried out to trace the biologically active principle led to the isolation of vestolide (**1**), which, however, was inactive The present paper is concerned with the structural elucidation of the latter

RESULTS AND DISCUSSION

Vestolide ($[M]^+$ *m/z* 282) displayed IR bands at 3400, 3325 (H-bonded hydroxyl groups), 1740 and 1660 cm^{-1} (α,β-unsaturated-γ-lactone) The presence of an exocyclic methylene group conjugated with the lactone was inferred from its UV maximum at 212 nm (ε12 408)

Since compound **1** was associated with another lactone of the same polarity only a small quantity could be isolated The two lactones were therefore resolved as their acetates The monoacetate of **1** ($[M]^+$ *m/z* 324) (**2**) was obtained as the major acetylated product The IR spectrum (KBr) of **2** also showed an intense band at 3425 cm^{-1} revealing the presence of hindered hydroxyl functions

The ^1H NMR spectrum of **2** exhibited two characteristic low-field doublets at δ5 34 (J = 3 Hz) and 6 14 (J = 3 5 Hz) corresponding to the protons of the exocyclic methylene γ-lactone Their J-values suggested *trans*-fusion of the lactone ring [1] The ^1H NMR spectrum also displayed two 3H singlets at δ1 22 and 1 45 corresponding to the two methyl groups present on carbons bearing hydroxyl groups The fact that **2** did not display any signal for a methyl group at the ring junction along with the requirement of a bicyclic nucleus suggested that it belonged to the guaianolide series The proton on the carbon bearing the oxygen of the lactone was responsible for a



R
1 H
2 Ac

*CDRI communication No 3377