Major Skin-Irritant Principle from *Synadenium grantii*

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Abstract □ The latex of *Synadenium grantii* Hook. f. (Euphorbiaceae) yielded a novel skin irritant, 12-*O*-tigloyl-4-deoxyphorbol-13-isobutyrate (I). The identity of the diterpene parent alcohol and the relative positions of the esterifying groups were established by spectral analysis and experiments leading to the hydrolysis and acetylation of I. Compound I is the first phorbol derivative to be reported from the genus *Synadenium*.

Keyphrases □ 12-*O*-Tigloyl-4-deoxyphorbol-13-isobutyrate—isolation as skin irritant from *Synadenium grantii* and identification □ Euphorbiaceae—isolation of phorbol ester derivative as skin irritant from *Synadenium grantii* □ Diterpene esters—isolation as skin irritant from *Synadenium grantii*

Synadenium grantii is a member of a small genus of 15 species indigenous to East Africa (1). The shrub is grown in Europe and North America as an ornamental plant (2). A previous clinical report described symptoms from contact with the latex of this plant, which included burning and blistering of the face and neck (3).

This study was initiated to determine whether the toxicological effects of *S. grantii* latex are due to phorbol ester constituents since many representatives of this class of irritant and tumor-promoting diterpenoids have been found in the related genus Euphorbia (4). A previous phytochemical study of *S. grantii* enabled the detection of euphol, tirucallol, euphorbol, and an isomer of lanosterol (5), although these phytosterols do not produce a skin-irritant effect (6).

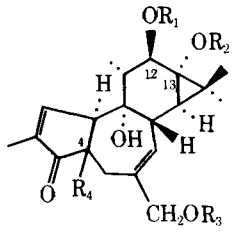
In the present work, fractionation of *S. grantii* latex resulted in the isolation of a novel irritant natural product, 12-*O*-tigloyl-4-deoxyphorbol-13-isobutyrate (I). Esters of 4-deoxyphorbol were isolated before from only two plant species (7–9).

EXPERIMENTAL

Plant Material—*Synadenium grantii* Hook. f. (Euphorbiaceae), propagated from a cutting[1], was used as the source of latex[2].

Extraction and Fractionation—Dried latex (19.9 g) was extracted at room temperature with six 100-ml portions of acetone. After drying, the residue (6.1 g) was partitioned between 60 ml of hexane and two 20-ml portions of methanol–water (9:1). Mouse ear-irritant activity (10) was traced to the methanol–water layer (1.1 g), which was subjected to low-pressure column chromatography on octadecylsilyl silica gel[3] with water–acetonitrile–methanol (3:2:2) as the eluting solvent. Irritant fractions were concentrated into 200 mg of the original column charge. The major active[4] principle, I, was purified by preparative TLC on silica gel G[3] in chloroform–benzene–ether–ethyl acetate (1:3:3:1) (R_f 0.22) and methylene chloride–ether–acetone (3:1:1) (R_f 0.55), as well as on diethylene glycol-impregnated kieselguhr G[3] (8) in cyclohexane–methyl ethyl ketone (9:1) (double development) (R_f 0.32).

Characterization of I—Resinous 12-*O*-tigloyl-4-deoxyphorbol-13-isobutyrate (I) (10 mg, 0.05%) exhibited major absorbance maxima in the IR spectrum[5] at ν_{max} 3430 (broad), 1710, 1650, 1625, and 1250 cm^{-1} and



I: R_1 = OCC(CH_3)=$CHCH_3$, R_2 = $OCCH(CH_3)_2$, R_3 = H, R_4 = βH
II: R_1 = OCC(CH_3)=$CHCH_3$, R_2 = R_3 = H, R_4 = αH
III: R_1 = R_2 = R_3 = $OCCH_3$, R_4 = αH

in the UV spectrum[6] at λ_{max} 220 (log ε 4.37) nm. The NMR spectrum[7] showed signals at δ 0.91 (d, 1H, J = 6 Hz, 14-H), 0.95 (d, 3H, J = 6Hz, 18-CH_3), 1.10–1.26 (m, 12H, 16-CH_3, 17-CH_3, and two isobutyl CH_3), 1.74 [m, 6H, 19-CH_3 and =$CH(CH_3)$], 1.85 (s, 3H, 2′-CH_3), 2.25–2.61 (m, 3H), 2.92 (m, 1H, OH, exchangeable with D_2O), 3.24 (m, 1H, 10-H), 3.99 (s, 2H, 20-H_2), 5.51 (d, 1H, J = 9.5 Hz, 12-H), 5.54 (m, 1H, 7-H), 5.82 (m, 1H, OH, exchangeable with D_2O), 6.91 [m, 1H, =$CH(CH_3)$], and 7.55 (m, 1H, 1-H). Its mass spectrum[8] exhibited principal fragment ions at *m/e* 500 (M^+, 4%), 482 (3), 457 (3; found, 457.2221; calc. for $C_{26}H_{33}O_7$, 457.2225), 412 (10), 401 (8), 312 (16; found, 312.1727; calc. for $C_{20}H_{24}O_3$, 312.1725), 294 (18), 83 (100), 71 (30), and 43 (52).

Hydrolysis, Epimerization, and Acetylation of I—Hydrolysis and epimerization of I (6 mg) in 0.02 *M* KOH in methanol for 15 min at room temperature (8) led to the formation of a more polar compound, 12-*O*-tigloyl-4-deoxy-4α-phorbol (II) (4 mg), which was purified by preparative TLC on silica gel[3] in methylene chloride–acetone–hexane (3:1:1) (triple development) (R_f 0.10). The major absorbances in the IR spectrum[5] of II were observed at ν_{max} 3400 (broad), 1690, 1640, and 1625 cm^{-1}, and in the UV spectrum[6] at λ_{max} 220 (log ε 3.82) nm. In the NMR spectrum[7] of II, an upfield shift for the C-1 proton to δ 7.10 was evident, as well as a downfield shift for the C-10 proton to δ 3.42, suggesting an epimerization of the C-4 proton on hydrolysis (7, 8). There were no signals in the NMR spectrum of II assignable to an isobutyrate moiety, although all of the tigliate signals observed in the NMR spectrum of I were present. Other assignable signals were observed at δ 0.95 (d, 3H, J = 8.8 Hz, 18-CH_3), 1.17 (s, 6H, 16-CH_3 and 17-CH_3), 1.79 (m, 3H, 19-CH_3), 3.92 (s, 2H, 20-H_2), and 4.91 (d, 1H, J = 12 Hz, 12-H). Although the molecular ion of II was not apparent in the mass spectrum, fragment ions were observed at *m/e* 394 (3%), 330 (2), 312 (16), 294 (14), 91 (27), 83 (100), 69 (24), and 55 (55).

Compound II (2 mg) was hydrolyzed further with 0.05 *M* KOH in methanol at ambient temperature for 45 min. The product was partitioned and acetylated by a standard procedure (8). The resulting compound, 4-deoxy-4α-phorbol-12,13,20-triacetate (III) (2 mg), was identical to an authentic sample (co-TLC, mass spectral, and NMR analyses) (8).

DISCUSSION

The parent diterpene alcohol of the major skin-irritant factor (I) in *S. grantii* latex was shown to be 4-deoxyphorbol by hydrolysis (with simultaneous epimerization) (7), acetylation, and direct comparison to 4-deoxy-4α-phorbol-12,13,20-triacetate (III). The two esterifying groups in I were positioned at C-12 and C-13 since the NMR resonance at δ 3.99

[1] Provided by Longwood Gardens, Kennett Square, Pa.
[2] The plant was identified by Dr. D. D. Soejarto, and a representative sample was deposited in the herbarium of the Field Museum, Chicago, Ill.
[3] E. Merck, Darmstadt, West Germany.
[4] Biological test data will be published later.
[5] Beckman 18-A (as potassium bromide pellet).
[6] Beckman DB-G (in methanol).
[7] Varian T-60A with Nicolet TT-7 FT attachment (60 MHz) (in deuterochloroform with tetramethylsilane as the internal standard).
[8] Hitachi Perkin-Elmer RMU-6D and Varian MAT 112S (70 ev, low resolution).

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is typical of other 4-deoxyphorbol diesters with a primary hydroxyl group at C-20 (7–9). Mass spectral and NMR observations permitted the assignment of these ester functions as tiglic and isobutyric acids (9, 11). The tiglic group was assigned to C-12 because a fragment ion in the mass spectrum of I at *m/e* 401 indicated the loss of an acyloxy radical rather than a whole acid substituent from the molecular ion, an effect that was noted previously in phorbol 12,13-diesters (12). In contrast, as indicated by the fragment ion at *m/e* 412, the C-13 isobutyric substituent left the molecule of I as an acid (12). Confirmation of these assignments was achieved using a selective hydrolysis procedure in which the C-12 substituent of I was less susceptible to hydrolysis than the C-13 substituent (8). This procedure resulted in the formation of II, in which the tiglyl substituent was detected by NMR and mass spectral analyses.

Esters of 4-deoxyphorbol appear to be rare in the plant kingdom, having been described previously in *Euphorbia tirucalli* (7, 8) and *E. biglandulosa* (9). The irritant principle I in *S. grantii* is the first representative in this series to be esterified with two short-chain acids.

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N-Demethylation of Dextromethorphan

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Abstract □ In addition to providing an efficient synthesis of 3-methoxymorphinan hydrochloride, the use of 2,2,2-trichloroethyl chloroformate in the *N*-demethylation of dextromethorphan led to the isolation of two novel zinc salts of 3-methoxymorphinan.

Keyphrases □ Dextromethorphan—*N*-demethylation to yield 3-methoxymorphinan hydrochloride and two novel zinc salts of 3-methoxymorphinan □ Antitussives—dextromethorphan, *N*-demethylation to yield 3-methoxymorphinan hydrochloride and two novel zinc salts of 3-methoxymorphinan □ 3-Methoxymorphinan—synthesis by *N*-demethylation of dextromethorphan

A sample of 3-methoxymorphinan (V) recently was required in these laboratories for pharmacological evaluation. Compound V is one of the three known metabolites of dextromethorphan (3-methoxy-*N*-methylmorphinan, I) (1, 2), a clinically useful antitussive agent. This report describes a new synthesis of V from I and the isolation of novel zinc salts of V.

EXPERIMENTAL[1]

Synthesis of 1,3,4,9,10,10a-Hexahydro-6-methoxy-2*H*-10,4a-(iminoethano)phenanthrene-11-carboxylic Acid 2,2,2-Trichloroethyl Ester (II)—Dextromethorphan hydrobromide monohydrate[2] (27.8 g, 75.0 mmoles) was partitioned between chloroform and a solution of 5.7 g of potassium hydroxide in water. The organic layer was separated, dried (sodium sulfate), and concentrated to leave a viscous oil, which solidified upon standing to yield 20.4 g (100%) of I, mp 107–110°; IR (mineral oil): 1610 cm^{-1}. TLC on silica gel with chloroform–methanol (9:1) gave a single spot at $R_f \simeq 0.1$.

To a solution of I (8.14 g, 30.0 mmoles) in 100 ml of benzene was added 2,2,2-trichloroethyl chloroformate[3] (6.99 g, 33.0 mmoles). After refluxing for 1 hr, the solution was concentrated to leave 14.6 g of crude II as a viscous oil; IR (mineral oil): 1710 (C=O) cm^{-1}; NMR (deuterochloroform): δ 4.70 (s, 2H, OCH_2); mass spectrum (70 ev, electron impact): *m/e* 431 (molecular ion). TLC on silica gel with chloroform–methanol (9:1) gave a single spot at $R_f \simeq 0.8$.

Synthesis of 3-Methoxymorphinan Tetrachlorozincate (IV)—To a solution of II (12.7 g, 29.3 mmoles) in 100 ml of 90% acetic acid was added 5 g of powered zinc. An exotherm followed. After 20 min, TLC indicated completeness of the reaction, and the mixture was filtered. The filtrate was concentrated to a white solid, which was triturated with ether and collected to yield 3-methoxymorphinan tetraacetatozincate (III), mp 157–167° (glass); NMR (deuterochloroform and dimethyl sulfoxide-d_6): δ 7.20–7.00 (m, 1H, aromatic), 6.87–6.68 (m, 2H, aromatic), 3.80 (s, 3H, OCH_3), 3.30–3.00 (m, 3H, $CHNHCH_2$), and 2.06 (s, ~6H, acetate ions); mass spectrum (70 ev, electron impact): *m/e* 257 (molecular ion). TLC on silica gel with chloroform–methanol (9:1) gave a single spot at $R_f \simeq 0.15$.

Crude III (10.0 g) was dissolved in 2000 ml of chloroform, and dry hydrogen chloride gas was bubbled through the solution for 10 min. The solution was concentrated, and the resulting oil partially crystallized upon standing under ether. Trituration with a small volume of isopropanol

[1] Melting points are uncorrected. IR spectra were recorded with a Perkin-Elmer model 727B spectrophotometer. NMR spectra were obtained with a Varian EM360A spectrometer. Mass spectra were recorded with a Finnigan model 4023 gas chromatograph–mass spectrometer (electron impact and chemical ionization) at 70 ev. Combustion analyses for carbon, hydrogen, nitrogen, chlorine, and zinc were performed by Dow Analytical Laboratories and Galbraith Laboratories, Knoxville, Tenn.

[2] Dow Chemical Co., Indianapolis, Ind.

[3] Aldrich Chemical Co., Milwaukee, Wis.

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