spectrometer equipped with a Digilab Fourier transform system. Single frequency off-resonance decoupling experiments were carried out with the proton decoupler at δ 14. Chemical shifts are reported in δ units (parts per million) relative to TMS ($\delta = 0$) as an internal standard in $CDCl_3$. All chromatographic separations were continuously assayed for UV absorption at 254 and 280 nm.

Isolation. Dried plants of *Chondrococcus hornemanni* (100 g) were collected at Trincomalee (Foul Point), Sri Lanka and extracted with Et_2O. The Et_2O extract (2.0 g) was applied to a 1 m × 1.5 cm Si gel column and eluted with hexane. Two main fractions were collected. Fraction 1 (520 mg), an oil, was rechromatographed on a 140 × 10 mm column of Si gel G. Elution with hexane afforded 270 mg (13.5%) of 3-bromomethyl-3-chloro-7-methyl-1,6-octadiene (**4**) as a colorless oil: $[\alpha]_D^{25} = -3.7°$ (CH_2Cl_2, $c = 14.69$); PMR δ 1.63 (*s*, allylic Me), 1.69 (*s*, allylic CH_3), 1.9–2.3 (complex *m*, C-4 CH_2 and C-5 CH_2), 3.68 (AB quartet, $\Delta\nu = 6.6$ Hz, $J_{gem} = 10.0$ Hz, $-CH_2Br$), 5.12 (broad *m*, C-6 CH), 5.21 (*dd*, $J_{cis} = 10.0$ Hz, $J_{gem} = 1.0$ Hz, C-1 H *cis* to C-2 H), 5.40 (*dd*, $J_{trans} = 16.5$ Hz, and $J_{gem} = 1.0$ Hz, C-1 H *trans* to C-2 H), 5.94 (*dd*, $J_{trans} = 16.5$ Hz, $J_{cis} = 10.0$ Hz, C-2 CH); CMR δ 138.6 (*d*, C-2), 132.6 (*s*, C-7), 122.5 (*d*, C-6), 116.9 (*t*, C-1), 72.4 (*s*, C-3), 40.1 (*t*, bromomethyl carbon), 39.2 (*t*, C-4), 25.6 (*q*, E-Me), 23.0 (*t*, C-5), 17.7 (*q*, Z-Me); IR (neat) 2960 (*s*), 2915 (*s*), 2850 (*s*), 1644 (*m*), 1440 (*s*), 1410 (*m*), 1379 (*m*), 1230 (*m*), 1105 (*m*), 980 (*s*), 929 cm^{-1} (*s*); MS *m/e* (rel. intensity) 250, 252, 254, (0.8:1:0.2 molecular ion cluster, <1), 215 (4), 217 (4), 135 (73), 93 (63), 91 (29), 69 (100), 41 (84). Fraction 2 (340 mg), a pale yellow waxy solid, was identified as a mixture of saturated fatty acids by PMR analysis and was not investigated further.

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A NEW CRYPTIC IRRITANT AND COCARCINOGEN FROM SEEDS OF *CROTON SPARCIFLORUS*

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Key Word Index—*Croton sparciflorus*; Euphorbiaceae; cryptic irritant; cryptic cocarcinogen; diterpene esters; 12-*O*-dodecanoylphorbol 13-acetate, 12-*O*-dodecanoylphorbol-13-acetate-20-linolenate.

Abstract—In recent years the genus Croton has been of particular interest following the isolation of 11 different 12,13-diesters of the polyfunctional diterpene phorbol (**1a**), from the seed oil of *Croton tiglium*; these compounds represent its toxic, irritant and cocarcinogenic principles [1]. In addition, croton oil contains what is called "cryptic" cocarcinogens of the phorbol-12,13,20-triester type [1]. The structurally related but non-irritant diterpene ester 20-acetoxy-9-hydroxy-13,15-seco-4α-tigliatriene-(1,6,14)-dione-(3,13) [2] and crotofolin A have been isolated from *C. rhamnifolius* [3] and from *C. corylifolious* L. [4], respectively.

In order to further study these irritant and cocarcinogenic compounds (croton factors), the seed oil of *Croton sparciflorus*, a herb native to Paraguay, was investigated. This plant is used in India as an antiseptic and styptic and is considered to be a troublesome weed [5]. Following the separation procedure developed for *Croton tiglium* seed oil coupled with the mouse ear irritation assay [1], a 12,13-diester and the corresponding 12,13,20-triester of phorbol were isolated, the latter representing a new "cryptic" irritant and cocarcinogen. Various alkaloids and other unrelated chemical constituents have been previously isolated from this species [5].

By comparison with *C. tiglium* seed oil (ID_{50}: 0.5 μg/ear [1]), the seed oil of *C. sparciflorus* is less irritant (ID_{50}: 17 μg/ear). By extracting the oil with methanol, which removes less irritant material as the hydrophobic fraction, the irritant activity is increased in the remaining hydrophilic fraction. On further extraction of the latter fraction with alkali, the activity is concentrated in the neutral fraction. By a Craig distribution [1] of this fraction further inactive material is removed to yield the active fraction, column chromatography of which gave an irritant and a less irritant fraction. From these fractions by PLC, two esters of phorbol, exhibiting different R_f and ID_{50} values were obtained. Similar differences in R_f values, and irritant activities are well known for phorbol-12,13-di- and phorbol-12,13,20-triesters [1]. From the NMR and mass spectra (molecular ion *m/e* = 588), the compound with the lower R_f is a phorbol ester containing a dodecanoic and acetic acid ester function. It has been reported [1] that in the MS of phorbol-12,13-diesters containing long and short chain fatty acyl residues, the long chain acid moiety is fragmented as a acyloxy-radical if it is in position 12. Also in such

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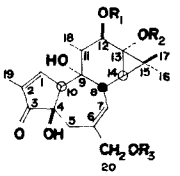
esters, a rounded IR band is observed at 1700 cm^{-1}. Both of these observations hold good in the case of the phorbol-diester isolated. Final confirmation of structure as a 12,13-diester of phorbol with the dodecanoic acid residue at C-12 and acetic acid at C-13, was obtained by comparing its IR spectrum with that of authentic croton oil factor A_3, 12-*O*-dodecanoylphorbol-13-acetate (**1b**) [1]; the spectra were superimposable. The cocarcinogenic activity of this phorbol-12,13-diester is well known [1].



(1a) $R_1 = R_2 = R_3 = H$

(1b) $R_1 = CO-(CH_2)_{10}-Me$, $R_2 = Ac$

(1c) R_1 and R_2 as in 1b

$R_3 = CO-CH_2-(CH_2)_5-(CH_2CH=CH)_3-CH_2-Me$

Comparison of the MS of the compound with $m/e = 588$ with that of the compound exhibiting the higher R_f indicates a mass difference of 260. However, the peaks due to the loss of dodecanoic acid ($m/e = 389$) and acetic acid ($m/e = 43$) are present in the spectrum. The mass difference can be accounted for if there is a third ester group containing a C_{18}-acid with three double bonds. The UV spectrum shows a very high extinction value (ϵ 33230) at λ_{max} 195 nm accounting for three isolated double bonds in the acid moiety. The IR spectrum exhibits a small peak at 3000 cm^{-1}, which is in accordance with the unsaturation in the chain [6]. The NMR spectrum of the compound is very similar to that of 12-*O*-dodecanoylphorbol-13-acetate. In addition it shows a large signal, equivalent to 28 protons at 1.28 ppm, a triplet at 2.8 ppm (4 protons) and a signal at 5.3–5.45 ppm (6 protons). The signal H_2-20 appearing in phorbol or in **1b** at 4.01 ppm is displaced to 4.43 ppm, suggesting that, as compared to **1b**, the compound contains a third ester function at C-20 of the phorbol moiety of **1b**. Based upon earlier experience [1] with phorbol-12,13,20-triesters from croton oil, a controlled acid catalysed transesterification was performed and the major product isolated by PLC. Its R_f value, mass, NMR and IR spectra are identical with those of 12-*O*-dodecanoylphorbol-13-acetate (**1b**). All data suggest that the compound is 12-*O*-dodecanoylphorbol-13-acetate-20-linolenate (**1c**).

Compared to the corresponding phorbol-12,13-diesters, phorbol-12,13,20-triesters show comparatively little irritant and cocarcinogenic activity. However the ester group in the 20-position may be selectively hydrolyzed to release the corresponding phorbol-12,13-diesters which are highly irritant and cocarcinogenic [1,7]. Whereas all the naturally occurring "cryptic" *Euphorbia* factors known to date have an acetoxy group at position −20 [e.g. 8,9], **1c** is the first such factor with a long chain unsaturated acid moiety at this site in the molecule.

EXPERIMENTAL

Plant material. Seeds of *Croton sparciflorus* Morong, originating from India, were supplied by Dr. E. L. Steinmetz, Amsterdam, The Netherlands.

General methods. Chemical shifts (60 Mhz) refer to tetramethylsilane ($\delta = 0.00$ ppm) as internal standard. For column chromatography Merck Si gel (0.05–0.20 mm, deactivated with 13% of water) and for PLC, Merck Si gel PF_{254}, is used. For visualizing the spots, the vanillin–H_2SO_4 reagent [10] was used. Plates, after being sprayed, were heated in an oven at 110°. In the standard procedure [1] the irritation unit (IU) on the mouse ear is measured to determine the correct dosing for measuring the irritant dose 50 (ID_{50}, level of significance $\alpha = 0.05$, standard deviation 6:1.3). For comparison of relative irritant activities, either IU's or ID_{50}'s have been used throughout (see also [11]).

Isolation and fractionation of seed oil: Crushed seeds (1 kg) were repeatedly extracted with peroxide free ether. Removal of ether gave the irritant seed oil (130 g, 13% yield, set 100% for separation procedure, IU: 100 μg/ear, ID_{50}: 17.2 μg/ear). The MeOH soluble irritant hydrophilic fraction (24.3 g, 18.6%, IU: 35 μg/ear, ID_{50}: 4.7 μg/ear) and the MeOH-insoluble hydrophobic fraction (104 g, 80.8%, IU: 680 μg) as well as the irritant neutral fraction (8.2 g, 6.3%, IU: 15 μg/ear, ID_{50}: 4.3 μg/ear) and the acid fraction (15.6 g, 12%, IU: 100 μg/ear) were prepared according to [11]. The neutral fraction was subjected to a Craig distribution (hand operated battery) in petrol-MeOH–H_2O (30:20:1) ($z = 5$, $n = 9$, $V = 50:50$, single withdrawal [1]), to give the inactive fraction (withdrawn fractions, $r = 5$ to 9, 6,5 g, 5.0%, IU: > 735 μg/ear) and the active fraction (fractions on battery, $r = 0$ to 4, 1.67 g, 1.28%, IU: 1.6 μg/ear, ID_{50}: 0.25 μg/ear). By column chromatography of the active fraction on deactivated Si gel, the irritant (500 mg, 0.38%, IU: 1.6 μg/ear, ID_{50}: 0.1 μg/ear) and the less-irritant fraction (1.1 g, 0.84%, IU: > 180 μg/ear) were eluted with Et_2O-petrol (1:1).

Isolation of 12-O-*dodecanoylphorbol*-13-*acetate* (**1b**). 500 mg the irritant fraction after PLC in Et_2O-petrol (3:1), gave 76 mg of **1b** with R_f 0.24 in $CHCl_3$–EtOAc (2:3) (0.06%, IU: 1.8 μg/ear, ID_{50}: 0.08 μg or 0.14 mμM/ear). MS: *m/e* (> 300), 588 (2.3%), 570 (2.4%), 546 (2.4%), 528 (2.5%), 510 (5.0%), 492 (5.0%), 389 (30%), 370 (8.8%), 346 (9.5%), 328 (95%), 310 (100%). In addition to the fragmentations recorded here, other fragments with low intensity (<10%) following the general formula $m/e = (M^+ - X) + 2$ were found in the spectrum. UV (MeOH): λ_{max}: 193 (ϵ 12380), 231 (ϵ5200), 325 nm (ϵ 140), IR (CH_2Cl_2): 3400, 1720, 1628 cm^{-1}. NMR ($CDCl_3$): H-1: 7.60 (*s*, broad), H-7: 5.70 (*d*, broad), H-12: 5.42 (*d*), H_2-20: 4.0 (*s*), H-8, H-10: 3.25 (*m*), H_2-5: 2.52 (*s*), $-CH_2CO$: 2.2–2.35, acetate: 2.1 (*s*), H_3-19: 1.76 (*m*), $-(CH_2)_9-$: 1.28 (*s*), OH (exchangeable with D_2O): 5.54 (*s*, OH-9).

Isolation of 12-O-*dodecanoyl*-13-O-*acetylphorbol*-20-*linolenate* (**1c**). PLC of 1.1 g of the less-irritant fraction, with Et_2O-petrol (3:1), yielded a viscous mass (340 mg), which after further PLC gave 280 mg of an oily mass R_f 0.37 in EtOAc–$CHCl_3$(2:3) (0.2%, IU: 50 μg/ear, ID_{50}: 3.4 μg or 4.0 mμM/ear). MS: *m/e*: 848 (0.1%), 788 (0.1%), 649 (0.1%), 630 (0.1%), 612 (2.3%), 588 (2.2%), 570 (2.2%), 492 (2.1%), 388 (2.2%), 370 (6.1%), 328 (25%), 310 (50%), 43 (100%), UV (MeOH): λ_{max}: 195 (ϵ33230), 228 (ϵ5230), 325 nm (ϵ90), IR (CH_2Cl_2): 3400, 3000, 1720, 1625 cm^{-1}, NMR ($CDCl_3$): H-1: 7.60 (*s*, broad), H-7: 5.70 (*d*, broad), 3-CH=CH–: (5.3–5.45), H_2-20: 4.43 (*s*), H-8, H-10: 3.23 (*m*), $2-CH_2-CH=CH-$: 2.8 (*t*), acetate: 2.08 (*s*), $-CH_2-CH=CH-$, CH_2CO: 2.1–2.6, 19-H_3: 1.78 (*m*), $-(CH_2)_{14}-$: 1.28 (*s*), OH (exchangeable with D_2O): 5.5 (*s*, OH-9).

Transesterification of **1c** *to* **1b**. 130 mg **1c** was treated in MeOH (50 ml) with 0.1 ml 70% $HClO_4$. After 24 hr at 4°, the reaction was stopped by addition of small amounts of NaOAc. PLC in EtOAc gave one major product, R_f 0.24 in $CHCl_3$–EtOAc (2:3) (60 mg). MS: *m/e*: 588 (0.2%), 570 (1.7%), 546 (2.5%), 528 (5.0%), 510 (6.5%), 492 (3.3%), 389 (7.3%), 370 (6.8%), 346 (9.5%), 328 (100%), 310 (95%), UV (MeOH): λ_{max}: 194 (ϵ11420), 231 (ϵ5200), 328 nm (ϵ75), IR (CH_2Cl_2): 3400,

1720, 1628 cm^{-1}. NMR ($CDCl_3$): H-1: 7.60 (*s*, broad), H-7: 5.70 (*d*, broad), H-12: 5.42 (*d*), H_2-20: 4.0 (*s*), H-8, H-10: 3.25 (*m*), H_2-5: 2.52 (*s*), $-CH_2CO$: 2.2–2.35, acetate: 2.1 (*s*), H_3-19: 1.76 (*m*), $-(CH_2)_6-$: 1.28 (*s*), OH (exchangeable with D_2O): 5.54 (*s*, OH-9).

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NEUE PIMARDIEN-DERIVATE AUS *OTHONNA*-ARTEN*

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Institut für Organische Chemie der Technischen Universität Berlin, Straße des 17. Juni 135, W. Germany

(*Eingegangen* 22 *December* 1975)

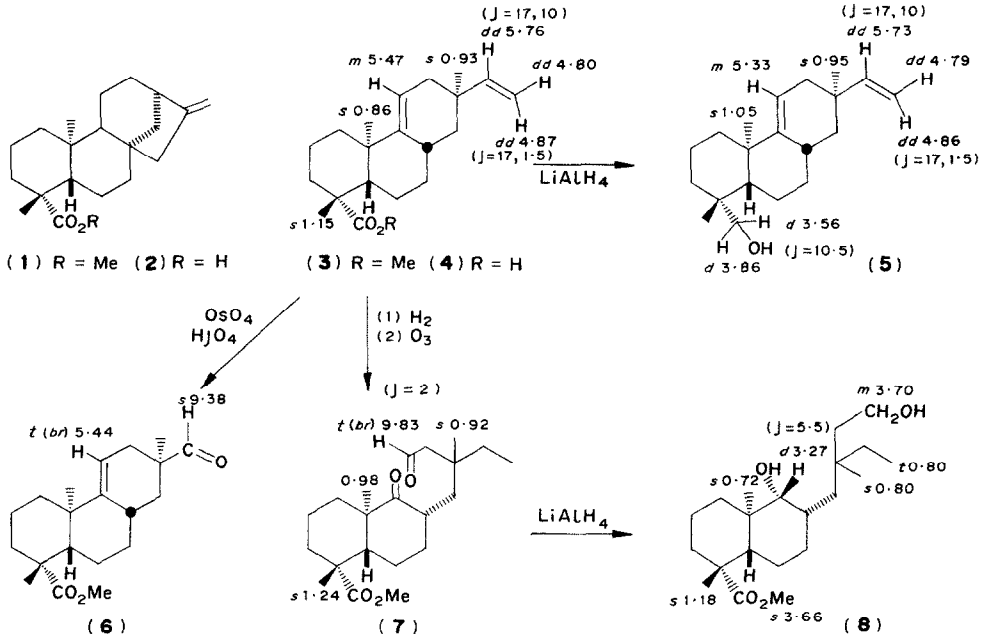
Key Word Index—*Othonna* species; Compositae; new diterpenes.

Pflanzen und Herkunft. Othonna cylindrica DC. und *O. floribunda* Schltr., Tribus Senecioneae, National Botanic Gardens Kirstenbosch, South Africa, Herbarbelege ebenda.

Bisherige Untersuchungen. O. cylindrica [1] Methylsalicylsäure-derivat. Die Gattung *Othonna* ist gekennzeichnet durch Furanoeremophilane [2,3].

O. cylindrica *DC.* Wurzel- und Blattextrakt enthalten keine Furansesquiterpene, dafür grosse Mengen an Diterpensäuren, die als Methylester durch Dünnschichtchromatographie trennbar sind. Neben **1** erhält man in etwa gleicher Menge ein Dien mit der Struktur **3**, so daß **2** und **4** als Naturstoffe vorliegen. Die Konstitution von **3** folgt aus den spektroskopischen Daten und denen der Abbauprodukte.

Mit Osmiumtetroxid/Perjodat erhält man den Aldehyd **6** und nach partieller Hydrierung und Ozonspaltung den Ketoaldehyd **7**, dessen NMR-Daten zusammen mit



* Mitt. 67 in Serie "Natürlich vorkommende Terpen-Derivate" Mitt 66 s Bohlmann, F. und Zdero, C. (1976) *Chem. Ber.* **109**, (im Druck).