

A NOVEL PHORBOL ESTER FROM EXCOECARIA AGALLOCHA¹

KAREN L. ERICKSON,² JOHN A. BEUTLER, JOHN H. CARDELLINA II, JAMES B. MCMAHON,

Laboratory of Drug Discovery Research and Development,

D. JOHN NEWMAN,

Natural Products Branch,

and MICHAEL R. BOYD*

Laboratory of Drug Discovery Research and Development, Developmental Therapeutics Program,
Division of Cancer Treatment, National Cancer Institute, NCI-FCRDC, Frederick, Maryland 21702-1201

ABSTRACT.—The novel phorbol ester 12-deoxyphorbol 13-(3*E*,5*E*-decadienoate) (**1**) was isolated as the anti-HIV principle of *Excoecaria agallocha* leaves and stems collected in northwest Australia. The structure was determined by spectral means. Compound **1** was also a potent displacer of [³H]-phorbol dibutyrate from rat brain membranes.

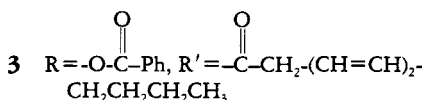
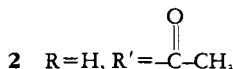
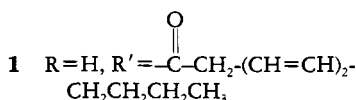
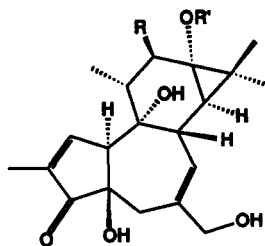
The widespread tropical genus *Excoecaria* (Euphorbiaceae) is known for the production of toxic metabolites. The leaves and latex of *Excoecaria agallocha* L. have been reportedly used as a source of dart-arrow and/or fish poison in Sarawak (2), New Caledonia (3), and Goa (4), while in Thailand they may appear in herbal preparations (5). In Pakistan, the latex has been used as a purgative and abortifacient and in the treatment of ulcers, rheumatism, leprosy, and paralysis (6). Excoecariatoxin, a piscicidal constituent of the twigs and bark of Okinawan *E. agallocha*, was characterized as a daphnane diterpenoid orthoester in 1974 (3). This same ester and related homologues were also found in the latex of *E. agallocha* native to Thailand (5) while taraxerane triterpenoids were reported from the leaves of a Hong Kong variety of the same species (7). In contrast to these terpenoid metabolites, a cinnamoyl piperidine was reported from the stemwood of an Indian specimen (4) and uncharacterized alcohols (presumably sterols) from the latex of a Pakistani sample (6).

We initiated an investigation of a northwestern Australian collection of *E.*

agallocha when extracts of the twigs and bark proved to be cytoprotective in the NCI primary anti-HIV screen (8). Additionally, the extracts displaced bound phorbol dibutyl ester (PDBu) from protein kinase C (PKC) (9).

Vlc of the crude extract (13.3 g) on Diol-60 columns, followed by two successive gel filtrations through Sephadex LH-20, hplc on silica, and, lastly, tlc on amino-coated silica plates afforded 4.1 mg of the active compound, **1**.

Hreims established the molecular formula of **1** as C₃₀H₄₂O₆, and its spectroscopy revealed the presence of OH (3416



¹Part 22 in the series "HIV-Inhibitory Natural Products." For part 21, see Rashid *et al.* (1).

²Visiting scientist on sabbatical from the School of Chemistry, Clark University.

TABLE 1. ^{13}C - (125 MHz) and ^1H - (500 MHz) Nmr Data for **1** (CDCl_3).

Carbon	^{13}C -Nmr ^a	^1H -Nmr ^b (m, Hz)	^1H - ^1H COSY
1	161.2	7.56 (dd, 2.4, 1.4)	10, 19
2	132.8	—	—
3	209.1	—	—
4	73.8	—	—
5	38.7	2.43 (d, 19.1) 2.49 (d, 19.1)	5b 5a, 7, 8, 10, 20a
6	139.8	—	—
7	130.3	5.65 (m)	5b, 7, 8, 20a, 20b
8	39.2	2.96 (dd, 5.4, 5.3)	5b, 7, 14
9	75.9	—	—
10	55.8	3.25 (dd, 2.9, 2.4)	1, 5b, 19
11	36.3	1.94 (ddq, 11.3, 6.9, 6.8)	12a, 12b, 18
12	32.2	1.54 (dd, 15.1, 11.7) 2.03 (m)	11, 12b 11, 12a, 18
13	63.8	—	—
14	32.5	0.82 (d, 5.4)	8
15	22.9	—	—
16	23.2	1.16 (s)	17
17	15.4	1.04 (s)	16
18	18.6	0.86 (d, 6.8)	11, 12b
19	10.1	1.76 (dd, 2.9, 1.4)	1, 10
20	68.3	3.97 (d, 12.7) 4.02 (d, 12.7)	5b, 7, 8, 20b 7, 8, 20a
21	173.8	—	—
22	38.3	3.06 (d, 6.9)	23, 24
23	121.3	5.56 (ddd, 15.1, 7.3, 6.9)	22, 24
24	134.6 ^c	6.08 (dd, 15.1, 10.3)	22, 23, 25
25	129.3	5.99 (dd, 15.1, 10.3)	24, 26
26	135.3 ^c	5.65 (m)	25, 27
27	31.8 ^d	2.03 (m)	26, 28
28	31.3 ^d	1.32 (m)	27, 29
29	22.2	1.29 (m)	28, 30
30	13.9	0.87 (t, 7.3)	29

^aAssignments based on comparison with values for prostratin (10) and yuanhuacin (16).

^bWith geminal protons, the smaller δ -value is given the a designation, the larger δ -value is given the b designation.

^{c,d}Values within a column may be interchanged.

cm^{-1}) and conjugated (1698 cm^{-1}) and unconjugated (1710 cm^{-1}) carbonyl groups. The ^{13}C - and ^1H -nmr spectral data, presented in Table 1, suggested a tiglane type of phorbol ester. Careful comparison of the nmr data of **1** with that of prostratin [**2**] (10) clearly identified **1** as a 12-deoxyphorbol ester. This was verified by ^1H - ^1H COSY nmr, which showed all of the expected correlations (Table 1) for the ring system and identified the esterifying acid moiety at C-13 as well. The latter contained four contiguous vinyl carbons each bearing a *trans*-related hydrogen ($J=15.1\text{ Hz}$). A termi-

nal butyl chain was attached to one end of the diene system while a methylene group at the other end connected the diene to the carbonyl carbon of the ester group. Thus, **1** is 12-deoxyphorbol-13-(3*E*,5*E*-decadienoate). This same esterifying group occurs in Wikstroemia factor C₁, which is phorbol 12-benzoate 13-(3*E*,5*E*-decadienoate) [**3**] (11).

Phorbol ester **1** is a potent in vitro inhibitor of HIV-1 replication as measured by inhibition of supernatant reverse transcriptase and p24 levels ($\text{IC}_{50}\ 6\text{ nM}$). It was not toxic to the host cells at concentrations up to the highest dose

tested, 2 μM , but was clearly cytostatic. The behavior of **1** in this regard is analogous to that of prostratin [**2**] (10). However, unlike prostratin, which does not appear to be a tumor promoter (10,12), **1**, with its hydrophobic decadienoate side-chain, may be expected to be an effective tumor promoter (13). Compound **1** potently displaced [^3H]-PDBu from rat brain membranes (IC_{50} 17 nM).

Compound **1** was the only PDBu- or anti-HIV-active metabolite apparent in the organic extract of this collection of *E. agallocha* bark and stem. This simplicity stands in contrast to the typical complexity of phorbol diterpene profiles in other Euphorbiaceae species. The occurrence in this case of a tigliane also differs from the previous isolation of daphnane orthoesters from this same species as piscicidal principles (3) and skin irritants (5).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—As reported previously (14).

EXTRACTION AND ISOLATION.—The bark, twigs, and leaves of *Excoecaria agallocha* L. (Euphorbiaceae) were collected at King George River in northwestern Australia in August 1991. Identification was made by Paul Dixon; a voucher sample has been deposited at the Smithsonian Institution, Washington, DC. The frozen plant material (800 g) was ground with dry ice, then the thawed mass was stirred for 4 h and filtered in a basket centrifuge. The marc was freeze-dried and extracted successively with CH_2Cl_2 -MeOH (1:1, v/v) and MeOH. The combined extracts were evaporated to yield an organic extract (14.4 g, 3.3% yield).

The crude extract (13.261 g) was chromatographed in batches by vlc on Diol-60 columns eluting successively with hexane, CH_2Cl_2 , EtOAc, Me_2CO , and MeOH. The active EtOAc fractions were pooled (2.097 g) and subjected to Sephadex LH-20 gel filtration with CH_2Cl_2 -MeOH (1:1) to give 405 mg of active material. Either a second Sephadex LH-20 column with hexane-toluene-MeOH (3:2:2) or Si gel vlc was then carried out, followed by hplc on silica with hexane-*i*-PrOH (9:1). Finally, prep. tlc on aminopropyl-bonded phase silica plates with hexane-*i*-PrOH (9:1) afforded 4.1 mg of **1** (0.03% from the crude extract).

12-Deoxyphorbol 13-(3E,5E-decadienoate) [**1**].— $[\alpha]_D^{25} +32.9^\circ$ ($c=0.2$, CHCl_3); uv (MeOH)

λ max 229 nm ($\epsilon=18,300$); ir (film) ν max 3416, 2924, 2872, 1710, 1698, 1628, 1378, 1332, 1244, 1171, 1134, 989, 757 cm^{-1} ; ^1H - and ^{13}C -nmr spectral data, see Table 1; eims m/z 498 [M^+] (8), 480 (6), 462 (1), 452 (1), 375 (59), 358 (28), 357 (100), 339 (37), 330 (23), 313 (33), 312 (86), 311 (44), 294 (53), 269 (26), 251 (21), 215 (29), 214 (28), 212 (27), 211 (41), 168 (28), 145 (28), 138 (31), 123 (53), 121 (37), 109 (37), 107 (39), 105 (27), 95 (33), 93 (28), 91 (36), 83 (81), 81 (56), 79 (52), 69 (38), 67 (97), 55 (36); hreims m/z 498.2974 (M^+ , calcd for $\text{C}_{30}\text{H}_{42}\text{O}_6$, 498.2970).

BIOASSAYS.—The anti-HIV screen was performed as previously reported (8), and the multiparameter assay as in (15). The PDBu binding assay was carried out as in (9).

ACKNOWLEDGMENTS

We thank Mary Koleck and Angela Constantinescu for PDBu binding assays, Glenn N. Gray for mass spectra, Robert Gulakowski and David Clanton for anti-HIV assays, Paul Dixon for taxonomic identification, Peter Murphy for the plant collection and helpful discussions, and Jason Mellica for technical assistance.

LITERATURE CITED

1. M.A. Rashid, K.R. Gustafson, Y. Kashman, J.H. Cardellina II, J.B. McMahon, and M.R. Boyd, *Nat. Prod. Lett.* **6**, 153 (1995).
2. T. Kawashima, T. Takahashi, Y. Inoue, M. Kodama, and S. Ito, *Phytochemistry*, **10**, 3308 (1971).
3. H. Ohigashi, H. Katsumata, K. Kawazu, K. Koshimizu, and T. Mitsui, *Agric. Biol. Chem.*, **38**, 1093 (1974).
4. S. Prakash, M.A. Khan, H. Khan, and A. Zaman, *Phytochemistry*, **22**, 1836 (1983).
5. P. Wiriyachitra, H. Hajiwangoh, P. Boonton, W. Adolf, H.J. Opferkuch, and E. Hecker, *Planta Med.*, **48**, 368 (1985).
6. M. Quadrat-I-Khuda, M.E. Ali, and M. Muslemuddin, *Sci. Res. (Dacca)*, **1**, 211 (1964).
7. W.H. Hui and M.L. Sung, *Aust. J. Chem.*, **21**, 2137 (1968).
8. O.S. Weislow, R. Kiser, D.L. Fine, J. Bader, R.H. Shoemaker, and M.R. Boyd, *J. Natl. Cancer Inst.*, **81**, 577 (1989).
9. J.A. Beutler, A.B. Alvarado, T.G. McCloud, and G.M. Cragg, *Phytother. Res.*, **3**, 188 (1989).
10. K.R. Gustafson, J.H. Cardellina II, J.B. McMahon, R.J. Gulakowski, J. Ishitoya, Z. Szallasi, N.E. Lewin, P.M. Blumberg, O.S. Weislow, J.A. Beutler, R.W. Buckheit, Jr., G.M. Cragg, P.A. Cox, J.P. Bader, and M.R. Boyd, *J. Med. Chem.*, **35**, 1978 (1992).

11. W. Dagang, B. Sorg, W. Adolf, H.J. Opferkuch, E.H. Seip, and E. Hecker, *Phytother. Res.*, **7**, 194 (1993).
12. Z. Szallasi, L. Krsmanovic, and P.M. Blumberg, *Cancer Res.*, **53**, 2507 (1993).
13. S. Zayed, B. Sorg, and E. Hecker, *Planta Med.*, **47**, 65 (1984).
14. Y.F. Hallock, K.P. Manfredi, J.W. Blunt, J.H. Cardellina II, M. Schaffer, K.-P. Gulden, G. Bringmann, A.Y. Lee, J. Clardy, G. François, and M.R. Boyd, *J. Org. Chem.*, **59**, 6349 (1994).
15. R.J. Gulakowski, J.B. McMahon, P.G. Staley, R.A. Moran, and M.R. Boyd, *J. Virol. Meth.*, **33**, 87 (1991).
16. B.-N. Zhou, L. Xue, L.-Z. Lin, L.-J. Lin, M.E. Johnson, and G.A. Cordell, *Magn. Reson. Chem.*, **31**, 194 (1993).

Received 6 October 1994