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IRRITANT PHORBOL DERIVATIVES FROM FOUR *JATROPHA* SPECIES

W ADOLF, H J OPFERKUCH and E HECKER

Deutsches Krebsforschungszentrum, Institut fur Biochemie, Im Neuenheimer Feld 280, 6900 Heidelberg, West Germany

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Key Word Index—*Jatropha curcas, J gossypifolia, J podagrica, J multifida*, Euphorbiaceae, diterpene esters, irritants, phorbol derivatives

Abstract—Four *Jatropha* species used in folk medicine were screened for irritant constituents By chromatographic and countercurrent distribution procedures, highly irritant factors were isolated from each species They represent new polyunsaturated esters of the tigliane-type diterpenoids 16-hydroxyphorbol (*J podagrica, J multifida*) and 12-deoxy-16-hydroxyphorbol (*J curcas, J gossypifolia*)

INTRODUCTION

Species of the genus *Jatropha* are known to be very toxic and the irritant and purgative activities of some of their seed oils are reminiscent of those shown by the toxic and irritant diterpene esters contained in the seed oils of many other Euphorbiaceae species, e g *Croton tiglium* [1] and *Euphorbia lathyris* [2] However, the chemical nature of the toxic principles of *Jatropha* species are unknown

Various plant parts of *J podagrica* are widely used in traditional folk medicine in West Africa [3, 4] Seeds and seed oil of *J multifida* and *J curcas* (physic nut, purge nut) are frequently used as purgatives in tropical countries [5–7] However, they may cause strong irritation and poisoning ('hell oil', 'oleum infernale', see refs [5–7]) In addition, the high toxicity of *J curcas* seeds to mice and goats has been demonstrated [8, 9] *J gossypifolia* is often used as a tea plant in Central America and its infusion is one of the most frequently used folk remedies of Curaçao [6] Its use may be related to the high incidence rates of oesophageal cancer on Curaçao [10] Extracts of the plant have also been used to treat cancerous growth [11] and the tumour inhibitory macrocyclic diterpene jatrophone was isolated from the roots [12] Further diterpenoid constituents, which may be biogenetically related [13], were inactive as tumour inhibitors [14]

We now report the isolation and chemical characterization of irritant diterpene esters from the seed oils of the four *Jatropha* species

RESULTS AND DISCUSSION

The seed oil of each of the *Jatropha* species was obtained by ether extraction of the homogenized seeds The fractionation procedure for the different seed oils was carried out as described for the seed oil of *Euphorbia lathyris* [2] Each fraction obtained was monitored by the assay for irritant activity on the mouse ear [1] As may be seen from Table 1, the seed oils were obtained in 20–40% yield and exhibited weaker irritant activity than the seed oil of *E lathyris* A 10–30-fold enrichment of the biological activity was achieved by preparation of the hydrophilic fractions, representing between 3 and 6% of the oils

Table 1 Yield and irritant activity* of the seed oils obtained from four *Jatropha* species and of corresponding subfractions obtained during separation procedures according to ref [2]

	Seed oil		Hydrophilic fraction†		Neutral fraction†	
Species	Yield (%)	ID_{50}^{24} (μg/ear)	Yield (%)	ID_{50}^{24} (μg/ear)	Yield (%)	ID_{50}^{24} (μg/ear)
J podagrica	40	>100	3 5	3 5	3 2	3 1
J multifida	34	70	3 2	2 5	2 0	4 4
J curcas	20	25	6 1	1 8	3 8	1 5
J gossypifolia	22	30	4 8	1 4	2 5	0 9
E lathyris	53	13	10 3	0 75	6 6	1 4

Determination of the irritant dose 50 (ID_{50}^{24}) according to ref [1] Standard Seed oil and corresponding fractions from *Euphorbia lathyris* [2]

*Yields and ID_{50}^{24} values of seed oils and fractions from *J curcas* and *J gossypifolia* are average values of three different fractionations, the other *Jatropha* species were fractionated only once

†Yields refer to seed oil = 100%

The corresponding hydrophobic fractions did not show any irritant activity and were discarded Acidic constituents were removed from the hydrophilic fractions by washing with sodium carbonate solution The neutral fractions thus obtained were further fractionated, in the case of *J podagrica* and *J multifida*, by column chromatography and in the case of *J curcas* and *J gossypifolia* by multi-stage Craig distribution

The irritant fractions obtained from both *J podagrica* and *J multifida* were shown to contain TLC homogeneous material with identical R_f values Whereas the material from *J podagrica* was characterized as the *Jatropha* factor P_1, the material from *J multifida* was separated by multiple development on TLC into two *Jatropha* factors, M_1 and M_2 (see Table 2), both exhibiting spectral data similar to the mixture (The UV data were similar for all *Jatropha* factors or materials, typical extinction values are presented for *Jatropha* factor M_1) The NMR data of factor P_1 and of factors M_1 and M_2 were very similar They suggested the presence of polyunsaturated acid esters (6–7 double-bond equivalents) of a common phorbol derivative Indeed, the same parent alcohol moiety (identical R_f value) was obtained from the three *Jatropha* factors by transesterification with 0 1 M sodium methanolate After acetylation of the parent alcohol, a tetraacetate was obtained exhibiting spectral data identical with those of an authentic sample of 16-hydroxyphorbol-12,13,16,20-tetraacetate (**2**, see Fig 1) 16-Hydroxyphorbol (**1**) is the tigliane-type parent alcohol of irritant and tumour-promoting factors from *Croton flavens* [15] and also of toxic constituents of *Aleurites fordii* [16] The exact chemical structures of the methyl esters obtained by transesterification of the mixture of M_1 and M_2 were not determined

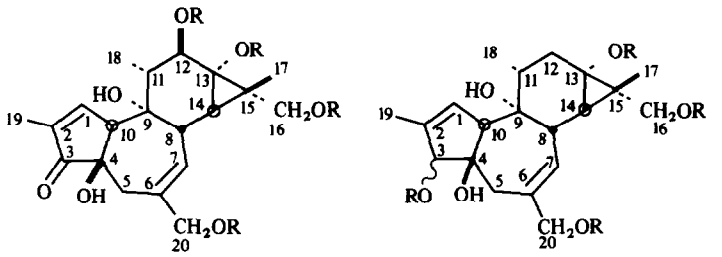
After multi-stage Craig distribution under identical conditions of the neutral fractions of *J curcas* and *J gossypifolia*, in both cases nearly all of the irritant activity was found in corresponding fractions According to TLC, they contained relatively uniform material They were finally purified by filtration through a small silica gel column, from both species irritant material was isolated exhibiting identical R_f values, which were, however, different from those of factors P_1, and M_1 and M_2 Multiple development of the apparently uniform material from both *Jatropha* species on TLC separated them into two factors, C_1 and C_2 (*J curcas*), and G_1 and G_2 (*J gossypifolia*), respectively (see Table 2) Attempts to separate the extremely unstable factors on a preparative scale were unsuccessful The NMR, UV and mass spectra of the pairs of *Jatropha* factors C_1/C_2 and G_1/G_2 were very similar, indicating the presence of esters of the same phorbol derivative with polyunsaturated acid moieties

The pairs of irritant factors isolated from *J curcas* and *J gossypifolia* did not react under the transesterification conditions described for the factors from *J podagrica* and *J multifida* When factors from *J curcas* and *J gossypifolia* were reduced with lithium aluminium hydride in diethyl ether followed by acetylation with acetic anhydride/pyridine, a product (**4**) (see Fig 1) was obtained exhibiting the same R_f value and spectral data as authentic 3-deoxo-12-deoxy-3ξ,16-dihydroxyphorbol-3,13,16,20-tetraacetate It is known that unsaturated 13-

Table 2 Yield, irritant activity and diterpene parents of various *Jatropha* factors

Jatropha species	*Jatropha* factors or mixtures	Yield (%)	ID_{50}^{24} (μg/ear)	Parent alcohol
podagrica	P_1	0013	007	16-Hydroxyphorbol
multifida	(M_1+M_2)	0011	005	16-Hydroxyphorbol
curcas	(C_1+C_2)	0013	002	12-Deoxy-16-hydroxyphorbol
gossypifolia	(G_1+G_2)	001	002	12-Deoxy-16-hydroxyphorbol

Standard 12-*O*-tetradecanoylphorbol-13-acetate (TPA), ID_{50}^{24} 0 02 μg/ear



1 R=H **3** R=H

2 R=COMe **4** R=COMe

Fig 1 Structure of 16-hydroxyphorbol (**1**) and its 12,13,16,20-tetraacetate (**2**), and of 3-deoxo-12-deoxy-3ξ,16-dihydroxyphorbol (**3**) and its 3,13,16,20-tetraacetate (**4**)

esters of 12-deoxy-16-hydroxyphorbol do not afford the parent alcohol under usual transesterification conditions [17] Thus it was concluded that the irritant principles of *J curcas* and *J gossypifolia* represent 12-deoxy-16-hydroxyphorbol-13-acylates with highly unsaturated acid moieties The exact chemical structure of the acid moieties was not elucidated in detail for *J curcas*, that of *J gossypifolia* is under way

It is noteworthy that the ID_{50} values of *Jatropha* factor P_1 and of all the mixtures of *Jatropha* factors isolated are comparable with that of 12-*O*-tetradecanoylphorbol-13-acetate (TPA, Table 2)

EXPERIMENTAL

Plant material Seeds of *J curcas* and *J gossypifolia* were purchased from F Steinmetz Co , Botanical Drugs, Amsterdam Seeds of *J podagrica* were a gift from Prof W Rauh, Dept of Botany, University of Heidelberg Seeds of *J multifida* were kindly provided by Dr J F Morton, Morton Collectanea, University of Miami, Florida

General methods TLC precoated silica gel plates PF_{254}, CC Merck silica gel (0 05–0 2 mm) deactivated with 13 % H_2O Spots on TLC were detected under UV light (254 nm) and/or by spraying with vanillin/H_2SO_4 For methods of countercurrent distribution, see ref [1] ^{1}H NMR 90 MHz, $CDCl_3$ with TMS as int standard

Assays for irritant activity Irritant doses 50 (ID_{50}^{24}) were determined on the mouse ear 24 hr after application according to the standard procedure [1] The standard deviations, σ, were all in the range 1 16–1 32, significance level $\alpha = 0\,05$

Preparation of seed oils The seeds (*J curcas*, 5 kg, *J gossypifolia*, 2 kg, *J multifida*, 2 kg, *J podagrica*, 500 g) were homogenized with an electric mill and extracted exhaustively with peroxide-free Et_2O Yields of seed oils and ID_{50} values are presented in Table 1

Fractionation procedures (for details see ref [2]) The seed oils were extracted several times with MeOH to yield inactive oily hydrophobic fractions ($ID_{50} > 100$ μg/ear) and active oily hydrophilic fractions (ID_{50} and yields, see Table 1) From the latter, by extraction with 1 M Na_2CO_3 the neutral fractions were obtained (Table 1)

Factors from J multifida *and* J podagrica On CC with Et_2O–petrol–Me_2CO (1 1 1), TLC-uniform material was obtained from the neutral fractions of the seed oils of *J podagrica* and *J multifida*, R_f 0 1 (EtOAc–petrol, 4 1) (for yields and ID_{50}^{24} see Table 2) Spectral properties of material from *J multifida* MS *m/z* 728, 726 $[M]^+$, UV λ_{max}^{MeOH} nm 222, 244, 254, 264 5, 275, 286, ^{1}H NMR (90 MHz, $CDCl_3$, TMS) δ7 6 (1H, *m*, H-1), 5 3–6 4 (15–16 olefinic H, 2 *m* (*br*) centred at 6 15 and 5 6, H-12 superimposed), 4 02 (2H, *s* (*br*), H_2-20), 3 84 (2H, *s* (*br*), H_2-16), 3 0–3 4 (5H, *m*), 2 5 (2H, *s* (*br*), H_2-5), 1 78 (3H, *m*, H_3-19), 5 23 (1H, *s*, OH-9, exchangeable with D_2O)

By multiple TLC the presence of two factors in *J multifida* was demonstrated The separation of irritant material (40 mg) into the factors M_1 (4 mg) and M_2 (2 mg) was achieved after developing 4 precoated plates (20 × 20 cm, 0 5 mm thickness) 7 times each in EtOAc–petrol (3 1) The mass spectra and NMR spectra were identical with those described for the mixture of *Jatropha* factors M_1 and M_2 UV λ_{max} nm (ε) factor M_1 (*m/z* 728) 216 (24 400), 244 sh (20 700), 254 sh (27 500), 264 (37 700), 274 (44 200), 285 (32 000), 324 (420), factor M_2 216, 244 sh, 254 sh, 264, 274, 285, 324

The mixture of M_1 and M_2 was treated with 0 1 M NaOMe–MeOH for 1 hr After adding buffer, pH 6 8, and extracting with *n*-BuOH, the resultant compound (**1**, R_f 0 08 in CH_2Cl_2–MeOH, 10 1) was acetylated (Ac_2O–pyridine) to yield **2** which was purified by TLC (Et_2O–petrol, 4 1, R_f 0 14) MS *m/z* 548 $[M]^+$, UV λ_{max}^{MeOH} nm (ε) 194 (14 600) and 254 nm (3000), ^{1}H NMR δ 7 58 (1H, *s* (*br*), H-1), 5 73 (1H, *d* (*br*), *J* = 6 Hz, H-7), 5 40 (1H, *d*, *J* = 10 Hz, H-12), 4 48 (2H, *s* (*br*), H_2-20), 4 27 (2H, AB, J_{AB} = 12 Hz, H_2-16), 3 1–3 3 (2H, *m*, H-8, H-10 superimposed), 2 45 (2H, *s* (*br*), H_2-5), 1 78 (3H, *m*, H_3-19), 0 93 (3H, *d*, *J* = 7 Hz, H_3-18), 5 4 (*s*, OH-9, superimposed with H_2-12), 3 92 (*s*, OH-4), 2 08–2 15 (12H, 4 acetates) All spectral data were identical with those of 16-hydroxyphorbol-12,13,16,20-tetraacetate (**2**) [15]

The factor P_1 isolated from *J podagrica* proved to be TLC-uniform after multiple development in various systems MS *m/z* 726 $[M]^+$, UV λ_{max}^{MeOH} nm 264, 275, 285, ^{1}H NMR δ7 56 (1H, *s* (*br*), H-1), 5 2–6 4 (*ca* 16 olefinic H), 4 0 (2H, *s*, H_2-20), 3 8 (2H, s (br), H_2-16), 2 5 (2H, *s* (*br*), H_2-5), 1 78 (3H, *m*, H_3-19)

Jatropha factor P_1 (2 mg) was treated with 0 1 M NaOMe–MeOH By TLC, the reaction product **1** was detected 1 hr later with R_f 0 08 in CH_2Cl_2–MeOH (10 1)

Factors from J curcas *and* J gossypifolia The neutral fractions from *J curcas* and *J gossypifolia* were each subjected to a Craig distribution in the system petrol–MeOH–H_2O (15 10 0 5, *z* = 500, *n* = 2160, *V* = 13 ml/10 ml) Irritant activity was found for both species in fractions *r* = 35–80, exhibiting in TLC one major product with R_f 0 21 (EtOAc–petrol, 2 1) and minor amounts of impurities on the base line and with higher R_f values The irritant material from both species was further purified by CC with the system above For yields and ID_{50} values of factors isolated see Table 2

The irritant material isolated from the two *Jatropha* species could be separated analytically into two factors (factor pairs C_1/C_2, G_1/G_2, see Table 2) when developed 3–4 times in EtOAc–petrol (2 1) Attempts to obtain a separation on a preparative scale failed

Spectral properties of the isolated material C_1/C_2 from *J curcas* MS *m/z* 710 $[M]^+$, UV λ_{max}^{MeOH} nm 195 5, 231, 274 (sh), 280, 287 (sh), 300 (sh), 316 5, ^{1}H NMR δ7 58 (1H, *m*, H-1), 4 5–6 5 (*ca* 14 olefinic H, H-7 superimposed), 4 0 (2H, *s*, H_2-20), 3 96 (2H, AB, 16-H_2), 2 8–3 3 (6H, including H-8 and H-10), 2 48 (2H, *s* (*br*), H_2-5), 1 78 (3H, *m*, 19-H_3), 5 27 (*s*, OH-9), 2 4 (*s*, OH-4)

Spectral data of the isolated material G_1/G_2 from *J gossypifolia* were identical (MS, NMR) with those of C_1/C_2 UV data showed identical λ values and similar ε values

Factors C_1/C_2 and G_1/G_2 did not react with 0 1 M NaOMe–MeOH, but when reduced with $LiAlH_4$–Et_2O (4 hr refluxing) a product (**3**) was obtained (R_f 0 1 in CH_2Cl_2–MeOH, 10 1) After acetylation with Ac_2O–pyridine and chromatography with Et_2O–petrol (1 1), compound **4** (R_f 0 18) was isolated from both species exhibiting identical spectral data MS *m/z* 534 $[M]^+$, IR ν_{max}^{KBr} cm^{-1} 3430, 1735, 1650, UV λ_{max}^{MeOH} (ε) nm 193 5 (20 000), 316 (100), ^{1}H NMR δ5 88 (1H, *m*, H-1), 5 72 (1H, *d* (*br*), H-7), 5 52 (1H, *m*, H-3), 4 5 (2H, *s*, H_2-20), 4 07 (2H, AB, 16-H_2), 3 1 (1H, *m*, H-8), 2 58 (2H, AB, H_2-5), 1 62 (3H, *m*, H_3-19), 4 95 (1H, *s*, OH-9), 2 05–2 20 (12H, 4 acetates) Spectral data identical with those of 3-deoxo-12-deoxy-3ξ,16-dihydroxyphorbol-3,13,16,20-tetraacetate (Gschwendt, M and Hecker, E , unpublished results)

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