Novel 12-Deoxy-16-hydroxyphorbol Diesters Isolated from the Seed Oil of *Jatropha curcas*

Wilhelm Haas, Heinz Sterk, and Martin Mittelbach*

Institute of Chemistry, Karl-Franzens-University Graz, Heinrichstrasse 28, 8010 Graz, Austria

Received February 20, 2002

Six unstable intramolecular diterpene esters were isolated from the seed oil of *Jatropha curcas*. Five of these, *Jatropha* factors C_2−C_6 (**3**−**7**), are new natural products, and the structure of the known *Jatropha* factor C_1 (**2**) has been revised. All compounds possess the same diterpene moiety, namely, 12-deoxy-16-hydroxyphorbol (**1**). The dicarboxylic acid moieties of **2**−**5** contain a bicyclo[3.1.0]hexane unit, and those of **6** and **7** a cyclobutane unit, which is described for the first time within this compound class. Compounds **4** and **5** are C-8′ epimers. The structures of **2**−**7** were elucidated by spectroscopic methods and give an insight into the biogenesis of the characterized substances.

Jatropha curcas L. (Euphorbiaceae) is an oil-bearing shrub widely distributed in many Latin American, Asian, and African countries.[1] The plant can be used in multiple ways, such as to prevent erosion, to reclaim land, to be grown as a live fence, or planted as a commercial crop, and therefore it has considerable potential to be cultivated on a large scale in the regions where it occurs.[2] The seed kernels, which contain up to 60% oil with a fatty acid composition similar to that of common edible oils,[3,4] are the most interesting parts of the plant from a potential commercial vantage point. However, the seeds and seed oil are toxic to humans and animals and for that reason nutritional utilizations are not possible.[5−11] A crucial obstacle in the establishment of *J. curcas* as a commercial crop would be overcome by detoxifying the seeds/seed oil.

The toxicity of the seeds of *J. curcas* is ascribed mainly to a group of diterpene esters termed the phorbol esters, which are contained in relatively high concentrations in the seeds of toxic *J. curcas* provenances, but only in low concentrations in the seeds of nontoxic *J. curcas* provenances from Mexico.[3,12] These substances are distributed in plant species of the families Euphorbiaceae and Thymelaeaceae, and their structure is based on a tetracyclic carbon skeleton known as tigliane.[13] They are known to cause a wide range of biological effects including tumor promotion and inflammation, and these adverse activities have been correlated to individual structural features[14−16] and have also been determined for the phorbol esters of *J.* curcas seeds/seed oil.[17,18] However, interest in these constituents of the seeds of *J. curcas* is restricted not only to the toxicity of the seeds. Molluscicidal activity of the seed extracts as well as widespread use of the seeds in traditional medicine may also be associated with the presence of these substances.[19−21] Chemical studies of *J. curcas* have led to the assumption that the seed oil contains up to four different phorbol esters.[22−25] Due to their low abundance and extreme instability, structure determination has so far been performed for only one of these compounds, which has been found to be an intramolecular diester of 12-deoxy-16-hydroxyphorbol.[18]

In the present paper the isolation and structure elucidation of six phorbol esters (**2**−**7**) from *J. curcas* seed oil is reported. Five of these (**3**−**7**) are novel natural products, and the structure proposed for the already known compound[18] mentioned above is revised (**2**). All isolated substances are intramolecular diesters of the same diterpene, 12-deoxy-16-hydroxyphorbol (**1**). The dicarboxylic acid part of two of the novel compounds (**6** and **7**) contains a cyclobutane unit, and therefore these components represent a new type of phorbol ester. The obtained results give an insight into the presumed biosynthesis of the characterized substances.

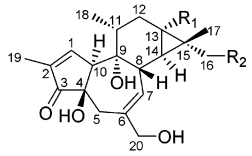
Results and Discussion

Compounds **2**−**7** were isolated by subjecting *J. curcas* seed oil to solid-phase extraction (SPE), repeated column chromatography, and repeated semipreparative HPLC. The structures of the compounds were elucidated on the basis of spectrosopic methods, including matrix-assisted laser desorption−ionization time-of-flight mass spectrometry (MALDI-TOF MS), and different NMR techniques inclusive of ^{1}H−^{1}H correlation (COSY), 1D and 2D total correlation (TOCSY), disortionless enhancement by polarization (DEPT), heteronuclear multiple quantum/bond coherence (HMQC/HMBC), and gradient-enhanced nuclear Overhauser effect (GOESY) NMR experiments. The chemical instability of the compounds, which could already be observed during isolation, turned out to be the most critical problem in determining the structures. However, decomposition could be hindered by storing the substances under argon.

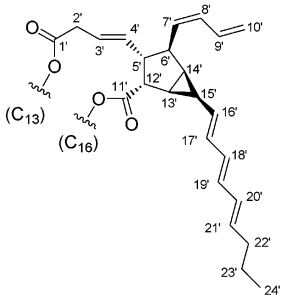
^{1}H NMR (see Tables 1 and 2) and ^{1}H−^{1}H COSY NMR data of compound **2** were almost identical to the previously published data of 12-deoxy-16-hydroxyphorbol-4′-[12′,14′-butadienyl]-6′-[16′,18′,20′-nonatrienyl]-bicyclo[3.1.0]hexane-(13-*O*)-2′-[carboxylate]-(16-*O*)-3′-[8′-butenoic-10′]-ate (DHPB),[18] except for small differences of the chemical shift values, which can be explained by the use of CD_2Cl_2 instead of $CDCl_3$[18] as solvent (decomposition of the compounds was reduced by using CD_2Cl_2). The signals assigned to the diterpene moiety were identical to literature data of 12-deoxy-16-hydroxyphorbol esterified at OH-13/OH-16 (**1**).[18,22] Assignment of the exchangeable proton signals at δ_H 1.59 (OH-20), 2.25 (OH-4), and 5.10 (OH-9) was based on a ^{1}H−^{1}H COSY correlation (H-20a,b/OH-20), respectively, from HMBC correlations and previously reported data (OH-4

* To whom correspondence should be addressed. Tel: +43 316 380 5353. Fax: +43 316 380 9841. E-mail: martin.mittelbach@kfunigraz.ac.at.

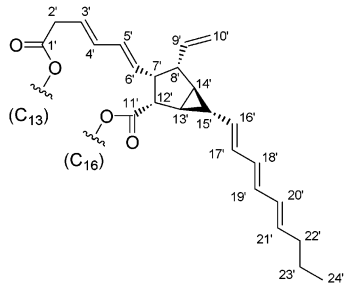
10.1021/np020060d CCC: $22.00
Published on Web 08/29/2002



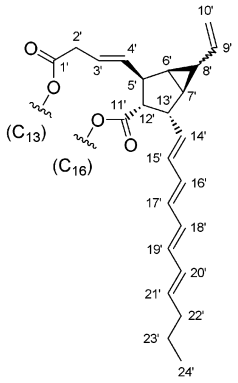
1 $R_1 = R_2 = OH$



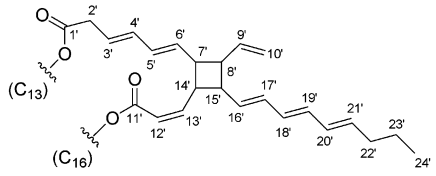
2



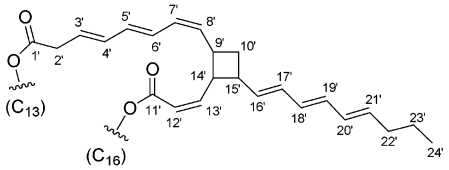
3



4,5



6



7

and OH-9).[22] The proposed stereochemistry of the diterpene moiety was confirmed by its characteristic NOE correlations (see Figure 1) and by comparison of published ^{1}H NMR chemical shifts;[26,27] the small chemical shift difference ($\Delta\delta_H$) of the two methylene protons H-5a (δ_H 2.48) and H-5b (δ_H 2,42) indicated the β-configuration for OH-4.[28] Detailed analysis of the ^{1}H NMR, ^{1}H$-^1$H COSY, TOCSY, and HMBC spectra enabled further confirmation of the published structure for the dicarboxylic acid residue (**2**).[18] Lack of ^{1}H$-^1$H COSY correlation between H-12′ (δ_H 3.18) and H-13′ (δ_H 1.78) indicated a nearly 90° dihedral angle between these two protons,[29] which was supported by molecular mechanics (MM2)[30] calculations (calculated angle: 86°).[31] NOE correlations between H-5′/H-12′, H-5′/H-16′, H-6′/H-4′, H-6′/H-9′, and H-6′/H-14′ (see Figure 2) corresponded to literature data on the relative stereochemistry of the bicyclo[3.1.0]hexane unit.[18] However, these previously reported results show only part of the stereochemical arrangement depicted in Figure 2. Prior studies on compound **2** led to the tentative assumption that C-1′ and C-11′ of the acid residue are connected to O-16 and O-13 of the diterpene moiety, respectively.[18] However, a weak HMBC correlation between C-11′ and H-16b revealed that this assignment had to be reversed; C-1′ is attached to O-13, and C-11′ to O-16. Therefore the correct structure of **2** was established as shown. Lack of NOE correlations between protons of the diterpene and the acid moiety prohibited the elucidation of their relative stereochemistry. The compound's instability prevented the assignment of its molecular weight using high-resolution electron impact mass spectrometry (HREIMS). MALDI-TOF MS gave an $[M + Na]^+$ at *m*/*z* 733.3662 (calculated for $C_{44}H_{54}O_8Na$, 733.3711), confirming the determined structure. According to the nomenclature used in a previously published work,[22] the revised compound **2** is termed *Jatropha* factor C_1 [indexing is based primarily on C_8 reversed-phase (RP)-HPLC retention times of the corresponding substances].

MALDI-TOF MS data of compound **3** showed the $[M + Na]^+$ ion at *m*/*z* 733.3775. The NMR data of **3** indicated that the diterpene moiety of this compound is 12-deoxy-16-hydroxyphorbol esterified at OH-13/OH-16. Therefore, **2** and **3** are isomers with structural differences restricted to the acid residues. Three methine protons at δ_H 1.70 (H-13′), 1.56 (H-14′), and 1.37 (H-15′), showing couplings to each other in the ^{1}H$-^1$H COSY spectrum, and the methine proton at δ_H 2.90 (H-7′), showing ^{1}H$-^1$H COSY cross-peaks to the methine protons at δ_H 2.82 (H-8′) and 3.04 (H-12′), suggested the presence of a bicyclo[3.1.0]hexane unit as a structural feature of the acid residue of **3**. This was corroborated by analysis of TOCSY and HMBC spectra. The missing ^{1}H$-^1$H COSY couplings between H-8′ and H-14′ and between H-12′ and H-13′ could again be explained by the corresponding dihedral angle values (MM2 calculated as 84° for H-8′/H-14′ and as 80° for H-12′/H-13′). Structural elucidation of the nonatrienyl residue (C-16′ to C-24′), the vinyl group (C-9′ to C-10′), and the hexadienoic ester chain (C-6′ to C-1′) attached to C-15′, C-8′, and C-7′, respectively, as well as the assignment of the ester carbonyl carbon C-11′ substituted at C-12′ was based on detailed analysis of ^{1}H$-^1$H COSY, TOCSY, and HMBC spectra. The HMBC data also displayed a correlation between C-11′ and H-16a and therefore revealed the location of the binding sites between the phorbol moiety and the dicarboxylic acid residue. The relative stereochemistry of the bicyclo unit of the acid moiety was established by NOE correlations between H-7′/H-5′, H-7′/H-15′, H-8′/H-10′, H-8′/H-15′, and H-12′/H-15′ (see Figure 2). The absolute configuration of the molecule could not be elucidated. Thus, the structure of the new compound **3**, *Jatropha* factor C_2, was determined to be as shown. Structural differences from *Jatropha* factor C_1 (**2**) are in the length of the carbon chain attached to C-6′(**2**)/C-8′(**3**) and in the length of the ester chain connecting C-5′(**2**)/C-7′(**3**) of the bicyclo unit with C-13 of the phorbol moiety, as well as in the configuration at C-6′(**2**)/C-8′(**3**). From these differences conclusions on the biosynthesis of the substances can be drawn, which will be discussed later.

The epimers **4** and **5** could not be separated by the chromatographic systems used. NMR data for both compounds were extracted from spectra of an approximately 3:1 mixture of **4** and **5** (see Tables 1 and 2). MALDI-TOF MS gave an $[M + Na]^+$ ion at *m*/*z* 733.3733. NMR data of the mixture indicated that both compounds share the same diterpene moiety as *Jatropha* factors C_1 (**2**) and C_2 (**3**). Analysis of 1D and 2D NMR spectra revealed that the gross structures of the dicarboxylic acid residues of **4** and **5** are identical. Analogous to the acid residue of **2**, this structure contains a bicyclo[3.1.0]hexane unit linked to the phorbol

Table 1. ^{1}H NMR Data for the Dicarboxylic Acid Moieties of Compounds **2**−**7** (δ, CD_2Cl_2 for **2**, **4**−**7**, $CDCl_3$ for **3**, 500 MHz)

position	**2**	**3**	**4**	**5**[a]	**6**	**7**
2′a	3.00 dd[b] (17.8, 6.1)[c]	3.17 dd (18.0, 5.8)	3.05 o		3.22 dd (18.9, 5.1)	3.17 dd (17.9, 7.8)
2′b	2.92 dd (17.8, 8.7)	3.08 dd (18.0, 9.6)	2.93 o		3.10 dd (18.9, 10.3)	3.12 dd (17.9, 7.1)
3′	5.46 ddd (15.1, 8.7, 6.1)	5.52 ddd (15.4, 9.6, 5.8)	5.30 o	5.26 o	5.53 ddd (15.0, 10.3, 5.1)	5.57 dt (14.2, 7.3)
4′	5.18 dd (15.1, 9.0)	5.95 dd (15.4, 9.8)	5.59 dd (15.1, 9.4)	5.62 dd (15.1, 9.6)	5.97 dd (15.0, 10.4)	6.04 o
5′	2.52 br q (9.0)	6.17 dd (15.5, 9.8)	3.11 br dt (4.2, 10.4)	3.26 br dt (4.2, 10.4)	5.89 dd (14.9, 10.4)	6.00−6.03 o[d,e]
6′	3.51 br dt (9.8, 5.3)	5.35 dd (15.5, 9.6)	1.52 o	1.65 o	5.62 dd (14.9, 9.5)	6.29 dd (13.9, 11.0)[e]
7′	5.34 o	2.90 br q (8.7)	1.33 dd (6.0, 2.9)	1.49 o	2.91 o	6.00−6.03 o[d]
8′	6.02 t (10.4)	2.82 dd (10.2, 7.7)	1.61 o	1.66 o	2.85 q (8.7)	5.71 dd (10.1, 8.2)
9′	6.67 dt (16.8, 10.4)	6.13 dt (16.9, 10.2)	5.36 o	5.99 o	6.25 ddd (16.9, 10.3, 8.7)	3.70 m
10′a	5.16 d (16.8)	5.02 dd (10.2, 2.2)	5.04 dd (17.2, 1.5)	5.36 o	5.19 dd (10.3, 1.7)	2.21 dt (11.5, 8.2)
10′b	5.09 d (10.4)	5.01 dd (16.9, 2.2)	4.85 dd (10.2, 1.5)	5.23 dd (11.8, 1.5)	5.14 dd (16.9, 1.7)	2.12 o
12′	3.18 d (9.0)	3.04 d (9.2)	2.38 dd (11.3, 7.2)	2.61 dd (11.1, 7.8)	5.75 dd (11.4, 1.3)	5.69 dd (11.4, 1.2)
13′	1.78 o	1.70 dd (6.4, 3.3)	3.04 o	2.98 o	6.27 dd (11.4, 9.3)	6.19 dd (11.4, 9.4)
14′	1.85 m	1.56 o	5.90 dd (15.0, 9.5)	6.00 o	4.57 br q (9.3)	4.32 br q (9.4)
15′	1.69 dd (8.8, 7.8)	1.37 dt (8.9, 3.3)	6.08 o		2.91 o	2.98 m
16′	5.68 dd (15.0, 7.8)	5.20 dd (14.9, 8.9)	6.05−6.25 o[d]		6.02 dd (14.5, 9.4)	5.77 dd (14.2, 7.1)
17′	6.33 m	6.10 m	6.05−6.25 o[d]		6.20 o	6.00−6.03 o[d]
18′	6.16 o	6.07 o	6.05−6.25 o[d]		6.17 o	6.00−6.03 o[d]
19′	6.16 o	6.07 o	6.05−6.25 o[d]		6.17 o	6.00−6.03 o[d]
20′	6.08 m	6.02 o	6.09 o		6.11 m	6.00−6.03 o[d]
21′	5.72 dt (14.5, 7.3)	5.66 dt (14.9, 7.2)	5.72 dt (14.9, 7.3)		5.75 dt (14.7, 7.4)	5.70 dt (14.2, 7.3)
22′	2.08 q (7.3)	2.06 q (7.2)	2.08 q (7.3)		2.10 q (7.4)	2.06 q (7.3)
23′	1.42 tq (7.3)	1.41 tq (7.2)	1.42 tq (7.3)		1.44 tq (7.4)	1.40 tq (7.3)
24′	0.91 t (7.3)	0.90 t (7.2)	0.90 t (7.3)		0.93 t (7.4)	0.89 t (7.3)

[a] Assignments are listed only for signals that differ from those of compound **4**. [b] Multiplicities are indicated by usual symbols; o, overlapped signal. [c] J in Hz. [d] The large number of partly overlapping signals at δ 5.9−6.3 (**4**) and δ 5.5−6.1 (**7**) prevented an exact assignment of these protons. [e]These assignments may be interchanged.

Table 2. ^{13}C NMR Data for the Dicarboxylic Acid Moieties of Compounds **2**−**7** (δ, CD_2Cl_2 for **2**, **4**−**7**, $CDCl_3$ for **3**, 125 MHz)[a]

position	**2**	**3**	**4**	**5**[b]	**6**	**7**
1′	173.3	173.7	173.5		173.8	173.9
2′	38.1	38.1	39.0		38.6	39.0
3′	125.0	121.8	122.0	122.3	123.3	122.1
4′	136.8	135.9	136.6	136.4	133.9	136.7
5′	51.7	132.6	49.9	51.7	129.8	129.9−132.3[c]
6′	45.0	128.9	28.6	26.8	131.6	130.6
7′	133.0	47.0	31.7	29.6	48.9	129.9−132.3[c]
8′	131.0	49.2	22.1	24.1	47.1	135.1
9′	133.1	139.4	139.8	135.1	137.7	35.7
10′	118.1	115.8	112.4	118.7	115.9	31.9
11′	175.2	174.8	173.9	174.6	166.6	166.3
12′	51.1	53.6	52.0	54.7	119.0	119.6
13′	26.8	30.0	45.4	44.3	145.9	153.3
14′	32.3	35.3	134.8	135.1	42.1	46.1
15′	23.3	24.4	130.3		44.1	43.4
16′	130.0	134.1	129−134[c]		132.0	136.4
17′	134.9	128.8	129−134[c]		130.4 or 132.7[c]	129.9−132.3[c]
18′	130.8	130.7	129−134[c]		130.4 or 132.7[c]	129.9−132.3[c]
19′	131.8	130.7	129−134[c]		130.4 or 132.7[c]	129.9−132.3[c]
20′	131.0	130.7	131.1		130.7	130.9
21′	135.8	134.6	135.9		135.6	135.3
22′	35.3	35.0	35.3		35.3	35.3
23′	22.9	22.6	22.8		23.0	23.0
24′	13.9	14.0	13.9		14.0	14.0

[a] Assignments are based on DEPT, HMQC, and HMBC experiments. [b] Assignments are listed only for signals that differ from those of compound **4**. [c] The large number of partly overlapping signals at δ 125−135 (**4**,**7**) and 130−135 (**6**) prevented an exact assignment of these carbons.

moiety by a butenoic ester chain (C-4′ to C-1′) and a single carbonyl ester carbon (C-11′). These are attached to adjacent carbon atoms (C-5′ and C-12′) of the five-membered ring of the bicyclo unit and connect this substructure to C-13 and C-16 of the phorbol moiety, respectively; the binding sites between the phorbol moiety and the dicarboxylic acid residue for **4** and **5**, respectively, were assigned in accordance with an HMBC correlation between C-11′ and H-16b. Apart from the length and position of the remaining carbon chains, the main structural difference within the acid residue of **2** versus those of **4** and **5** consists of the orientation of the bicyclo[3.1.0]hexane unit, relative to the phorbol moiety. In the structure of **2**, one of the bridgehead carbons (C-13′) is adjacent to the carbon, which bears the single carbonyl ester carbon as a substituent. In compounds **4** and **5**, these two positions (C-7′ and C-12′) are separated by a carbon atom of the five-membered ring.

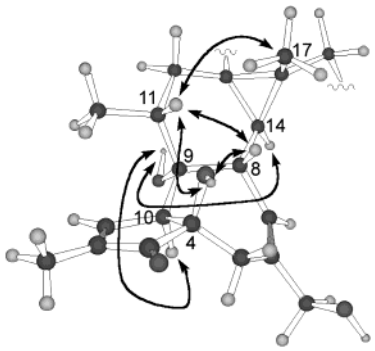


Figure 1. Selected NOE correlations of the 12-deoxy-16-hydroxyphorbol moiety of **2**−**7**.

The lack of ^{1}H−^{1}H COSY correlations between H-7′ and H-13′ observed for **4** and **5** was again explained by the corresponding dihedral angle values (MM2 calculated as 79° and 80° for **4** and **5**, respectively). Further structural units of the acid residue of **4** and **5**, respectively, are an undecatetraenyl residue (C-14′ to C-24′) attached to C-13′ and a vinyl group substituted at C-8′. The partial overlap of most of the olefinic methine proton signals (see Table 1) complicated the determination of the ^{1}H−^{1}H coupling constants, and therefore the configuration of some of the

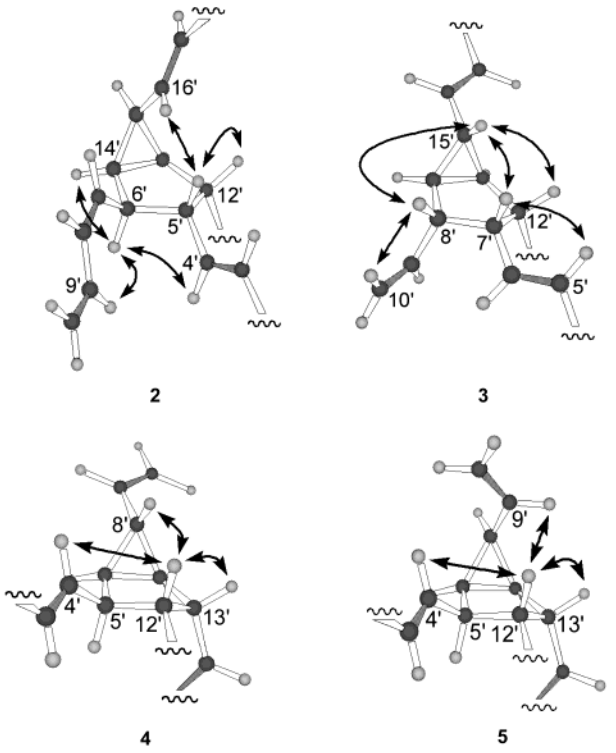


Figure 2. Key NOE correlations of parts of the dicarboxylic acid residues of **2**−**5**.

double bonds may be reversed. ^{1}H NMR data of the acid moiety of **5** showed close similarity to those of the acid moiety of **4** with the exception of downfield shifts of H-4′, H-5′, H-6′, H-7′, H-8′, H-9′, H-10′a, H-10′b, H-12′, and H-14′ and upfield shifts of H-3′ and H-13′ (see Table 1). Chemical shift differences were also observed for the corresponding carbons, including the carbonyl ester carbon C-11′ (see Table 2). From the fact that these effects were displayed only for signals assigned to the bicyclo system or to protons/carbons, which are adjacent to this system, different configurations of the bicyclo[3.1.0]hexane units of **4** and **5** may be inferred. This was confirmed by NOE correlations between H-8′/H-12′ of **4** and H-9′/H-12′ of **5** (see Figure 2), demonstrating that the new compounds **4** and **5** are C-8′ epimers; **4** (*Jatropha* factor C_4) adopts the 8′*S* configuration, whereas **5** (*Jatropha* factor C_5) shows the 8′*R* configuration. It is worth mentioning that the resonances of three protons of the phorbol moiety are also influenced by the two different configurations at C-8′ (OH-9: δ_H 5.15 for **4**, 5.16 for **5**; H-16a: δ_H 4.18 for **4**, 4.19 for **5**; H-16b: δ_H 3.57 for **4**, 3.54 for **5**). The effect on H-16a and H-16b can be attributed to the fact that these protons are adjacent to the bicyclo system. The influence on the hydroxylic proton OH-9 may be explained by hydrogen bonding to the carbonyl oxygens of C-1′ and C-11′. This is corroborated by comparison with similar structures, where the low-field shift of the OH-9 proton is supposed to result from hydrogen bonding of this type.[32]

The residual relative stereochemistry of the bicyclo system was found to be the same for both substances, as shown by NOE correlations between H-12′/H-4′ and H-12′/H-13′ and by the lack of any NOE correlation between H-12′/H-5′ (see Figure 2), which was complemented by the ^{1}H−^{1}H coupling constants of H-12′ (dd; J = 11.3, 7.2 Hz assigned to coupling with H-5′ and H-13′, respectively, for **4**; J = 11.1, 7.8 Hz assigned to coupling with H-5′ and H-13′, respectively, for **5**). The absolute stereochemistry of **4** and **5** could not be determined.

On the basis of MALDI-TOF MS and NMR data, it was determined that the molecular formula as well as the diterpene moiety of compound **6** is identical to those of **2**−**5**. However, NMR data of the acid residue showed differences (see Tables 1 and 2). In addition to a methyl signal at δ_H 0.93 (CH_3-24′), three methylene signals at δ_H 1.44 (CH_2-23′), 2.10 (CH_2-22′), 3.22, and 3.10 (H-2′a and H-2′b, respectively), and four methine signals at δ_H 2.91 (H-7′ and H-15′), 2.85 (H-8′), and 4.57 (H-14′), the ^{1}H NMR and HMQC spectra exhibited solely olefinic signals. The three methine signals, which appeared in the ^{1}H NMR spectra of **2**−**5** within a chemical shift range of δ_H 1.30−1.90 and were assigned to protons of the three-membered ring of the bicyclo[3.1.0]hexane unit, could not be observed in the ^{1}H NMR spectrum of **6**. This indicated that no bicyclo unit is included in the structure of the acid residue of this compound. ^{1}H−^{1}H COSY cross-peaks between H-7′/H-8, H-8′/H-15′, H-14′/H-7′, and H-14′/H-15′ as well as coupling of each one of the protons H-7′, H-8′, H-14′, and H-15′ with an olefinic signal led to the structure of the cyclobutane unit as shown in **6**. Ambiguities in assignments, which result from the fact that the signals of H-7′ and H-15′ have identical proton shifts (δ_H 2.91), were overcome by detailed analysis of the TOCSY and HMBC spectra. Structure elucidation of the carbon chains attached to C-8′ and C-15′ and of the ester chains linking the cyclobutane carbons C-7′ and C-14′ to C-13 and C-16, respectively, of the phorbol moiety was performed on the basis of NMR data. An HMBC correlation between C-11′ and H-16a revealed the location of the sites of connectivity between the diterpene moiety and the dicarboxylic acid residue. It is to be noted that the configuration of the double bond between C-12′ and C-13′ was determined as *Z* based on ^{1}H−^{1}H couplings (H-12′, dd, J = 11.4, 1.3 Hz assigned to coupling with H-13′ and H-14′, respectively; H-13′, dd, J = 11.4, 9.3 Hz assigned to coupling with H-12′ and H-14′, respectively), while an *E* configuration was proposed for the remaining double bonds. Identical ^{1}H NMR chemical shifts δ_H 2.91 of H-7′ and H-15′ hindered the determination of the relative stereochemistry of the cyclobutane unit on the basis of NOE experiments. The *cis* and *trans* configurations of vicinal cyclobutane protons are typified by only small differences between the corresponding ^{1}H−^{1}H coupling constants.[29] Therefore, determination of the stereochemical arrangement of this unit could not be achieved by analysis of ^{1}H−^{1}H couplings, and for that reason, the depicted structure of the new compound **6**, *Jatropha* factor C_3, does not include any information on the relative configuration.

The instability of compound **7** exceeded those of the other *Jatropha* factors obtained. Decomposition and the small amount of this isolated substance limited the number of experiments that could be performed. Thus, no NOE data could be measured to obtain information on the relative stereochemistry of **7**. The molecular formula of **7**, which was obtained from MALDI-TOF MS and NMR data, was identical to those of compounds **2**−**6**. NMR data were in close similarity to those of **6**, with the exception that the ^{1}H NMR data of the dicarboxylic acid residue of **7** included only three aliphatic methine proton signals at δ_H 3.70 (H-9′), 4.32 (H-14′), and 2.98 (H-15′) instead of the four signals displayed in the ^{1}H NMR spectrum of **6** and assigned to the cyclobutane protons. Furthermore, the ^{1}H NMR spectrum of **7** exhibited additional signals of two nonequivalent aliphatic methylene protons at δ_H 2.21 (H-10′a) and 2.12 (H-10′b); the signals of two olefinic protons at δ_H 5.19 (H-10′a) and 5.14 (H-10′b) that appeared in the ^{1}H NMR spectrum of **6** were missing. Detailed analysis of 1D and

2D NMR spectra led to the determination of the structure of the acid moiety of **7**. Differences from the structure of the acid moiety of **6** consist in a tri- rather than tetrasubstituted cyclobutane unit and in the length of the ester chain leading from the cyclobutane unit to C-13. Assignment of the connection sites between the acid residue and the phorbol moiety was based on an HMBC correlation between C-11′ and H-16a. The configurations of the double bonds between C-12′ and C-13′ and between C-7′ and C-8′ were assigned as *Z*, and those for all other double bonds as *E*. The corresponding $^1H-^1H$ coupling constants were partly extracted from overlapped signals, and therefore the assignments of the configuration of some of the double bonds may be reversed. Nevertheless, a hypothesis corresponding to the biosynthesis of the *Jatropha* factors (see below) favors the configurations depicted. Thus, the structure of the new compound **7**, *Jatropha* factor C_6, can be described as shown. Neither the absolute stereochemistry of the molecule nor the relative configuration of the cyclobutane unit could be assigned.

The small number of published studies on the characterization of intramolecular phorbol diesters includes hypotheses on the formation of these structures. The cyclohexene unit contained in the dicarboxylic acid moiety of the *Anthostema* factor S_5, as well as the bicyclo[3.1.0]-hexane substructure of the dicarboxylic acid moieties of DHPB [revised as compound **2** (*Jatropha* factor C_1) in the present paper] and of the structurally similar pycnocomolides, is assumed to be formed from two originally separated monoacid groups, of which at least one is attached to the phorbol moieties.[18,32−34] It is supposed that the cyclohexene unit is generated by a secondary Diels−Alder condensation.[18] The lack of good precedent permits only speculations on the kind of reactions leading to the generation of the bicyclo[3.1.0]hexane unit. However, the complexity of the substructure indicates a presumably enzyme-mediated formation.[32]

Knowledge of the structures of *Jatropha* factors C_1-C_6 (**2**−**7**) leads to the assumption that all these intramolecular diesters have been built up from two originally separated monoester groups and therefore confirms the hypothesis cited above. The specific monoacid groups may be supposed to be the same for all compounds: (*E*,*E*,*Z*)-3,5,7,9-decatetraenoic acid attached to OH-13 of the phorbol moiety and (*Z*,*E*,*E*,*E*,*E*)-2,4,6,8,10-tetradecapentenoic acid attached to OH-16 of the phorbol moiety (see Figure 3). The dicarboxylic acids of **6** and **7** are most likely generated by a (2+2) cycloaddition of the two monoacid groups. The following carbon atoms of the monoacids are incorporated into the cyclobutane unit: C-14′/C-15′ of the tetradecapentaenoic acid in **6** and **7**, and C-7′/C-8′ and C-9′/C-10′ of the decatetraenoic acid in **6** and **7**, respectively (see Figure 3). It is highly speculative to discuss the reactions leading to the formation of the bicyclo[3.1.0]hexane unit in the dicarboxylic acid moieties of **2**−**5**. However, the structures indicate that one of the two hypothetically monoacid groups contributes two carbons to the bicyclo system, while the other one contributes four carbons. In **2** and **3** the four carbons (C-12′ to C-15′) are provided by the tetrapentaenoic acid. The positions of the two carbons of the decatetraenoic acid involved into the formation of the bicyclo system are C-5′/C-6′ in **2** and C-7′/C-8′ in **3** (see Figure 3). It is noteworthy that the presumed relative configuration of the corresponding double bonds in the monoacid groups (*E* for C-5′/C-6′, *Z* for C-7′/C-8′) influences the relative stereochemistry of the bicyclo[3.1.0]hexane unit. An *E* configuration of the double bond in the monoacid group (C-5′/C-6′ in the hypothetical precursor of **2**; see Figure 3) leads to a *trans* configuration at the formed C−C bond in the bicyclo system (C-5′/C-6′ in **2**). The same rule can be applied for the reversed configuration (**3**). In **4** and **5** the decatetraenoic acid provides the four carbons to the bicyclo[3.1.0]hexane unit. This is displayed by a different orientation of the bicyclo system by comparison with **2** and **3**. It is again speculative to discuss the rationale for the formation of the two C-8′ epimers. A presumable explanation may be the conformational flexibility of the hypothetical precursor monoacid group based on the methylene carbon adjacent to the carboxylic carbon. As assumed, the *Z* configuration of the double bond between the two carbons (C-12′/C-13′) provided to the bicyclo system by the tetradecapentaenoic monoacid group leads to a *cis* configuration at the C−C bond formed (C-12′/C-13′ in **4** and **5**).

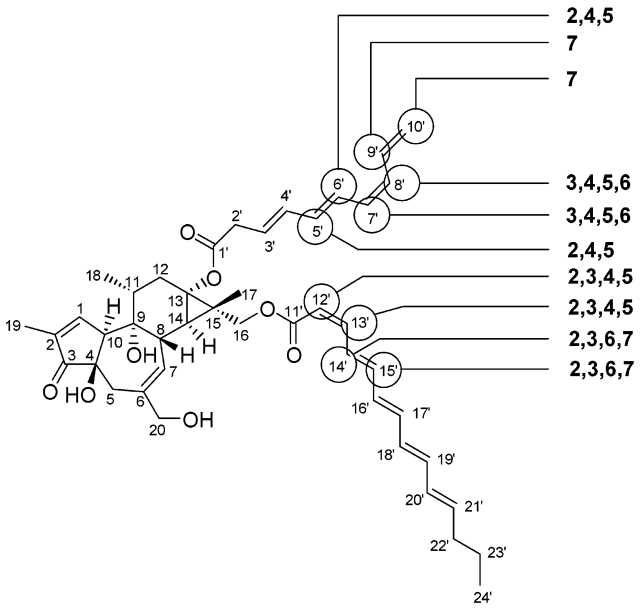


Figure 3. Hypothetical precursor of **2**−**7**. Carbons, which are assumed to be involved into the formation of the individual dicarboxylic acid residues, are assigned to the according compounds.

Experimental Section

General Experimental Procedures. Optical rotations were measured in MeOH on a Perkin-Elmer 241 polarimeter. UV spectra were obtained on a Shimadzu UV-2101PC spectrophotometer. ^1H NMR spectral data and $^1H-^1H$ COSY, $^1H-^1H$ TOCSY, DEPT, HMQC, HMBC, selective 1D $^1H-^1H$ TOCSY, and GOESY experiments were recorded in CD_2Cl_2 or $CDCl_3$ on a Bruker DRX-500 spectrometer with standard pulse sequences operating at 500 and 125 MHz for ^1H and ^{13}C, respectively. Chemical shifts are reported in δ values with respect to solvent signals (CD_2Cl_2: δ_H 5.32, δ_C 54.00; $CDCl_3$: δ_H 7.27, δ_C 77.23). Samples for NMR experiments were generally degassed and sealed under argon. MALDI-TOF MS were measured on a Shimadzu/Kratos AXIMA-CFR spectrometer using a nitrogen laser (337 nm) on 2,5-dihydroxybenzoic acid as matrix. Solid-phase extraction (SPE) and open column chromatography were carried out over silica gel (40−63 μm, Merck). HPLC was performed on a HP 1100 Series HPLC system coupled to a HP variable-wavelength detector set at 280 nm. Monitoring of the fractions obtained by SPE, open column chromatography, and HPLC was carried out by TLC [precoated plates, silica gel 60 F_{254}, 0,2 mm, Merck; $CH_2Cl_2-CH_3CN$ (7:3) as eluent]. TLC visualization was done with a UV lamp (254 nm) and by spraying with 1% vanillin in H_2SO_4 followed by heating the plates.

Plant Material. *Jatropha curcas* seed oil was provided by Proyecto Biomasa, Universidad Nacional de Ingenieria, RU-

PAP, Managua, Nicaragua. The oil was obtained by using an expeller press from seeds, which were collected in August 2000 from plants cultivated on an agricultural test area of the Universidad Nacional de Ingenieria, RUPAP, Managua, Nicaragua. The plant material was identified by Alfredo Grijalva, Universidad Centroamericana, Managua, Nicaragua. A voucher specimen (MM 1) of the seed oil is stored at −24 °C at the Institute of Chemistry, University of Graz.

Extraction and Isolation. A solution of 3.09 kg of seed oil in 4.5 L of petroleum ether was divided into 12 equal portions. Each of them was applied separately onto a SPE column, which had previously been packed with 12 g of silica gel and preconditioned with 50 mL of petroleum ether. After sample application, the columns were washed successively with petroleum ether (250 mL) and CH_2Cl_2 (250 mL), and the crude extracts comprising compounds **2**−**7** were eluted with MeOH (50 mL), combined, and concentrated in vacuo (40 °C).

The viscous brownish residue obtained (7.73 g) was divided into two equal portions, which were separately subjected to open column chromatography over silica gel [300 g for each of both separations, CH_2Cl_2−CH_3CN (7:3) used as eluent]. The resultant fractions were monitored by TLC, and the residue (3.52 g) from those containing compounds **2**−**7** (all compounds exhibited identical R_f values of 0.17 and were colored gray after spraying with vanillin−H_2SO_4 reagent and heating) was rechromatographed twice over silica gel (180 g) using CH_2Cl_2−acetone (4:1) and CH_2Cl_2−CH_3CN (7:3), respectively. A portion (1.12 g) of the residue (2.16 g) was further purified using silica gel HPLC [LiChrospher Si 60, 250 × 10 mm, particle size 10 μm, Merck; CH_2Cl_2−2-propanol (87:13), flow rate 7 mL/min] to afford an amorphous white powder (115.7 mg) comprising compounds **2**−**7**, which were eluted as two broad poorly separated peaks (t_R 7.2 and 8.2 min). Isolation of the individual compounds was performed by subjecting the mixture to C_8 reversed-phase HPLC [Spherisorb S5 Octyl, 250 × 16 mm, particle size 5 μm, Waters; CH_3CN−H_2O (75:25), flow rate 7 mL/min], yielding compounds **2** (t_R 12.2 min), **3** (t_R 13.2 min), **6** (t_R 14.2 min), and a combination of compounds **4**, **5**, and **7** (t_R 15.8 min). For further purification, the four fractions were further chromatographed by HPLC [LiChrospher Diol, 250 × 4 mm, particle size 5 μm, Merck; hexane−CH_2Cl_2−2-propanol (65:31:4), flow rate 1 mL/min] to afford **2** (15 mg, t_R 14.2 min), **3** (6 mg, t_R 11.0 min), **6** (3 mg, t_R 11.9 min), **7** (1 mg, t_R 9.4 min), and a mixture of **4** and **5** (7 mg, t_R 9.9 min).

***Jatropha* factor C_1 (2):** amorphous white powder; $[\alpha]^{20}_D$ +20.9° (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ϵ) 284 (4.69) nm; ^{1}H NMR data of the 12-deoxy-16-hydroxyphorbol moiety (CD_2Cl_2, 500 MHz) δ 7.57 (1H, s, H-1), 5.60 (1H, d, J = 4.7 Hz, H-7), 5.10 (1H, s, OH-9), 4.30 (1H, d, J = 11.8 Hz, H-16a), 4.02 (1H, d, J = 13.1 Hz, H-20a), 3.97 (1H, d, J = 13.1 Hz, H-20b), 3.57 (1H, d, J = 11.8 Hz, H-16b), 3.24 (1H, br s, H-10), 2.97 (1H, overlapped, H-8), 2.48 (1H, d, J = 19.2 Hz, H-5a), 2.42 (1H, d, J = 19.2 Hz, H-5b), 2.25 (1H, s, OH-4), 2.12 (1H, dd, J = 7.1, 15.1 Hz, H-12a), 1.98 (1H, m, H-11), 1.75 (3H, overlapped, CH_3-19), 1.60 (1H, dd, J = 11.4, 15.1 Hz, H-12b), 1.59 (1H, overlapped, OH-20), 1.19 (1H, s, CH_3-17), 1.18 (1H, d, J = 6.0 Hz, H-14), 0.88 (3H, d, J = 6.4, CH_3-18); ^{13}C NMR data of the 12-deoxy-16-hydroxyphorbol moiety (CD_2Cl_2, 125 MHz, based on DEPT, HMQC, and HMBC experiments) δ 208.7 (C-3), 161.2 (C-1), 140.9 (C-6), 133.4 (C-2), 129.5 (C-7), 76.4 (C-9), 74.0 (C-4), 74.0 (C-16), 68.4 (C-20), 63.4 (C-13), 56.1 (C-10), 39.1 (C-5), 38.9 (C-8), 36.7 (C-11), 33.4 (C-14), 32.0 (C-12), 25.9 (C-15), 18.7 (C-18), 12.0 (C-17), 10.1 (C-19); HMBC correlations of the 12-deoxy-16-hydroxyphorbol moiety (CD_2Cl_2) C-1→H-19; C-2→H-1, 19; C-3→H-1, 5a, 5b, 19; C-4→H-1, 5a, 5b, OH-4, 9; C-5→H-7; C-6→H-5a, 5b, 8, 20a, 20b; C-7→H-5a, 5b, 8, 14, 20a, 20b; C-8→H-7; C-9→H-5a, 8, 10, 12a, 18, OH-9; C-10→H-1, 5a, OH-4, OH-9; C-11→H-12a, 12b, 18; C-12→H-11, 18; C-13→H-12a, 12b, 14, 16a, 17, 18; C-14→H-7, 8, 12a, 16b, 17; C-15→H-8, 12a, 12b, 14, 16a, 16b, 17; C-16→H-14, 17; C-17→H-14, 16a, 16b; C-18→H-12b; C-19→H-1; C-20→H-5b, 7; ^{1}H and ^{13}C NMR data of the dicarboxylic acid moiety, see Tables 1 and 2; HMBC correlations of the dicarboxylic acid moiety (CD_2Cl_2) C-1′→H-2′; C-2′→H-3′, 4′; C-3′→H-2′, 5′; C-4′→H-2′, 5′; C-5′→H-3′, 4′, 12′, 13′, 14′; C-6′→H-5′, 8′, 12′; C-7′→H-5′, 8′; C-8′→H-10′a, 10′b; C-9′→H-7′, 8′, 10′a; C-10′→H-8′; C-11′→H-5′, 12′, 13′, 16b; C-12′→H-5′; C-13′→H-12′; C-14′→H-12′; C-15′→H-12′, 16′; C-17′→H-18′, 19′; C-18′→H-16′, 19′; C-19′→H-21′; C-20′→H-19′, 22′; C-21′→H-22′, 23′; C-22′→H-21′, 23′, 24′; C-23′→H-22′, 24′; C-24′→H-22′, 23′; ESIMS *m*/*z* 733 [M + Na]$^+$; HR-MALDI-TOF MS *m*/*z* 733.3662 [M + Na]$^+$ (calcd for $C_{44}H_{54}O_8Na$, 733.3711).

***Jatropha* factor C_2 (3):** amorphous white powder; $[\alpha]^{20}_D$ +0.6° (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ϵ) 280 (4.86) nm; ^{1}H NMR data of the 12-deoxy-16-hydroxyphorbol moiety ($CDCl_3$, 500 MHz) δ 7.58 (1H, m, H-1), 5.60 (1H, br d, J = 5.6 Hz, H-7), 5.44 (1H, s, OH-9), 4.81 (1H, d, J = 11.2 Hz, H-16a), 4.03 (1H, dd, J = 5.6, 12.9 Hz, H-20a), 3.98 (1H, dd, J = 6.4, 12.9 Hz, H-20b), 3.28 (1H, br s, H-10), 3.13 (1H, d, J = 11.2 Hz, H-16b), 3.06 (1H, overlapped, H-8), 2.53 (1H, d, J = 19.2 Hz, H-5a), 2.46 (1H, d, J = 19.2 Hz, H-5b), 2.22 (1H, d, J = 0.90 Hz, OH-4), 2.13 (1H, dd, J = 7.0, 14.9 Hz, H-12a), 2.00 (1H, m, H-11), 1.78 (3H, dd, J = 1.2, 2.8 Hz, CH_3-19), 1.60 (1H, overlapped, H-12b), 1.43 (1H, overlapped, OH-20), 1.08 (3H, s, CH_3-17), 0.94 (1H, d, J = 5.2 Hz, H-14), 0.89 (3H, d, J = 7.4, CH_3-18); ^{13}C NMR data of the 12-deoxy-16-hydroxyphorbol moiety ($CDCl_3$, 125 MHz, based on DEPT, HMQC, and HMBC experiments) δ 208.8 (C-3), 161.2 (C-1), 140.4 (C-6), 133.0 (C-2), 129.4 (C-7), 75.9 (C-9), 73.6 (C-4), 68.7 (C-16), 68.2 (C-20), 64.3 (C-13), 55.7 (C-10), 38.8 (C-5), 38.3 (C-8), 36.3 (C-11), 31.7 (C-12), 30.0 (C-14), 26.1 (C-15), 18.4 (C-18), 10.6 (C-17), 10.2 (C-19); HMBC correlations of the 12-deoxy-16-hydroxyphorbol moiety were consistent with those of **2**; ^{1}H and ^{13}C NMR data of the dicarboxylic acid moiety, see Tables 1 and 2; HMBC correlations of the dicarboxylic acid moiety ($CDCl_3$) C-1′→H-2′a, 2′b, 3′, 4′; C-2′→H-3′, 4′; C-3′→H-2′a, 2′b, 5′; C-4′→H-2′a, 2′b, 5′, 6′; C-5′→H-3′, 4′, 7′; C-6′→H-4′, 7′; C-7′→H-5′, 6′, 13′, 14′; C-8′→H-6′, 7′, 9′, 10′a, 10′b, 12′, 13′, 14′, 15′; C-9′→H-7′, 8′, 10′b, 14′; C-10′→H-8′; C-11′→H-7′, 12′, 13′, 16a; C-12′→H-5′, 6′, 7′, 8′, 13′, 14′, 15′; C-13′→H-8′, 12′, 15′, 16′; C-14′→H-8′, 9′, 10′b, 12′, 13′, 15′, 16′; C-15′→H-8′, 12′, 16′, 17′; C-16′→H-12′, 13′, 14′; C-17′→H-15′; C-21′→H-22′, 23′; C-22′→H-20′, 21′, 23′, 24′; C-23′→H-21′, 22′, 24′; C-24′→H-22′, 23′; ESIMS *m*/*z* 733 [M + Na]$^+$; HR-MALDI-TOF MS *m*/*z* 733.3775 [M + Na]$^+$ (calcd for $C_{44}H_{54}O_8Na$, 733.3711).

***Jatropha* factors C_4 and C_5 (4 and 5):** amorphous white powder; $[\alpha]^{20}_D$ +113.0° (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ϵ) 290 (4.68), 303 (4.88), 317 (4.83) nm; ^{1}H NMR data of the 12-deoxy-16-hydroxyphorbol moiety (CD_2Cl_2, 500 MHz) δ 7.55 (1H, m, H-1), 5.59 (1H, d, J = 4.2 Hz, H-7), 5.16 (1H, s, OH-9 of **5**), 5.15 (1H, s, OH-9 of **4**), 4.19 (1H, d, J = 11.9 Hz, H-16a of **5**), 4.18 (1H, d, J = 11.9, H-16a of **4**), 4.00 (1H, dd, J = 6.0, 12.8 Hz, H-20a), 3.95 (1H, dd, J = 6.0, 12.8 Hz, H-20b), 3.57 (1H, d, J = 11.9 Hz, H-16b of **4**), 3.54 (1H, d, J = 11.9, H-16b **5**), 3.24 (1H, br s, H-10), 2.95 (1H, overlapped, H-8), 2.47 (1H, d, J = 19.1 Hz, H-5a), 2.41 (1H, d, J = 19.1 Hz, H-5b), 2.34 (1H, s, OH-4), 2.09 (1H, overlapped, H-12a), 1.96 (1H, m, H-11), 1.74 (3H, dd, J = 1.2, 2.7 Hz, CH_3-19), 1.67 (1H, t, J = 6.0 Hz, OH-20), 1.54 (1H, overlapped, H-12b), 1.16 (1H, d, J = 6.0, H-14), 1.16 (3H, s, CH_3-17), 0.87 (3H, d, J = 6.5, CH_3-18); ^{13}C NMR data of the 12-deoxy-16-hydroxyphorbol moiety (CD_2Cl_2, 125 MHz, based on DEPT, HMQC, and HMBC experiments) δ 208.9 (C-3), 161.0 (C-1), 140.8 (C-6), 133.3 (C-2), 129.3 (C-7), 76.2 (C-9), 74.2 (C-4), 73.9 (C-16), 68.2 (C-20), 63.4 (C-13), 55.9 (C-10), 38.8 (C-5), 38.8 (C-8), 36.5 (C-11), 33.2 (C-14), 31.9 (C-12), 25.5 (C-15), 18.5 (C-18), 12.1 (C-17), 10.2 (C-19); HMBC correlations of the 12-deoxy-16-hydroxyphorbol moiety were consistent with those of **2**; ^{1}H and ^{13}C NMR data of the dicarboxylic acid moiety, see Tables 1 and 2; HMBC correlations of the dicarboxylic acid moiety (CD_2Cl_2) [correlations of compound **5** are only listed when observed for signals, which differ from those of compound **4** (see Tables 1 and 2)] C-1′→H-2′a, 2′b, 4′; C-2′→H-3′, 4′; C-3′→H-2′a, 2′b; C-4′→H-2′a, 2′b, 5′, 12′; C-5′→H-3′, 4′, 7′, 12′, 13′ (**4**); C-5′→H-4′, 7′, 12′, 13′ (**5**);C-6′→H-4′, 5′, 13′; C-7′→H-13′, 14′; C-8′→H-9′, 10′a, 10′b (**4**); C-8′→H-7′, 10′a, 10′b (**5**);C-9′→H-7′, 10′a; C-11′→H-5′, 12′, 16b (**4**); C-11′→ H-12′, 16b (**5**); C-12′→H-7′ (**4**); C-12′→ H-7′ (**5**); C-13′→H-12′, 14′, 15′ (**4**); C-13′→H-7′, 12′, 14′ (**5**); C-14′→H-12′, 13′ (**4**); C-14′→H-12′, 13′ (**5**); C-15′→H-13′; C-20′→H-22′;

C-21′→H-22′, 23′; C-22′→H-21′, 23′, 24′; C-23′→H-21′, 22′, 24′; C-24′→H-22′, 23′; ESIMS *m/z* 733 $[M + Na]^+$; HR-MALDI-TOF MS *m/z* 733.3733 $[M + Na]^+$ (calcd for $C_{44}H_{54}O_8Na$, 733.3711).

***Jatropha* factor C_3 (6):** amorphous white powder; $[\alpha]^{20}_D$ +130.0° (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ϵ) 272 (4.74) nm; ^{1}H NMR data of the 12-deoxy-16-hydroxyphorbol moiety (CD_2Cl_2, 500 MHz) δ 7.55 (1H, br s, H-1), 5.59 (1H, d, J = 5.2 Hz, H-7), 5.38 (1H, s, OH-9), 4.14 (1H, d, J = 11.6 Hz, H-16a), 4.00 (1H, dd, J = 5.5, 13.0 Hz, H-20a), 3.95 (1H, dd, J = 6.2, 13.0 Hz, H-20b), 3.52 (1H, d, J = 11.6 Hz, H-16b), 3.24 (1H, br s, H-10), 3.05 (1H, overlapped, H-8), 2.48 (1H, d, J = 19.2 Hz, H-5a), 2.43 (1H, d, J = 19.2 Hz, H-5b), 2.28 (1H, d, J = 0.7 Hz, OH-4), 2.12 (1H, dd, J = 7.1, 15.0 Hz, H-12a), 1.98 (1H, m, H-11), 1.74 (3H, dd, J = 1.3, 2.8 Hz, CH_3-19), 1.62 (1H, dd, J = 11.7, 15.0 Hz, H-12b), 1.62 (1H, br t, J = 5.9 Hz, OH-20), 1.15 (3H, s, CH_3-17), 0.96 (1H, d, J = 5.1 Hz, H-14), 0.88 (3H, d, J = 6.5, CH_3-18); ^{13}C NMR data of the 12-deoxy-16-hydroxyphorbol moiety (CD_2Cl_2, 125 MHz, based on DEPT, HMQC, and HMBC experiments) δ 208.7 (C-3), 160.8 (C-1), 140.7 (C-6), 133.1 (C-2), 129.1 (C-7), 76.0 (C-9), 74.0 (C-4), 69.4 (C-16), 68.2 (C-20), 64.4 (C-13), 55.9 (C-10), 38.9 (C-5), 38.5 (C-8), 36.5 (C-11), 31.9 (C-12), 30.2 (C-14), 26.3 (C-15), 18.6 (C-18), 10.9 (C-17), 10.2 (C-19); HMBC correlations of the 12-deoxy-16-hydroxyphorbol moiety were consistent with those of **2**; ^{1}H and ^{13}C NMR data of the dicarboxylic acid moiety, see Tables 1 and 2; HMBC correlations of the dicarboxylic acid moiety (CD_2Cl_2) C-1′→H-2′a, 2′b; C-2′→H-4′; C-3′→H-2′a, 2′b, 5′; C-4′→H-2′a, 2′b; C-5′→H-3′, 7′/15′; C-6′→H-7′/15′, 8′, 14′; C-7′→H-5′, 6′, 8′, 9′, 14′, 15′; C-8′→H-7′/15′, 9′, 10′a, 10′b, 16′; C-9′→H-7′/15′, 8′, 10′b; C-10′→H-8′; C-11′→H-12′, 13′, 16a; C-12′→H-14′; C-13′→H-7′/15′, 8′, 12′, 14′; C-14′→H-7′/15′, 8′, 12′; C-15′→H-8′, 14′, 13′, 16′; C-16′→H-7′/15′, 14′; C-20′→H-22′; C-21′→ H-22′, 23′; C-22′→H-20′, 21′, 23′, 24′; C-23′→H-21′, 22′, 24′; C-24′→H-22′, 23′; ESIMS *m/z* 733 $[M + Na]^+$; HR-MALDI-TOF MS *m/z* 733.3559 $[M + Na]^+$ (calcd for $C_{44}H_{54}O_8Na$, 733.3711).

***Jatropha* factor C_6 (7):** amorphous white powder; $[\alpha]^{20}_D$ +69.3° (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 276 (5.11) nm; ^{1}H NMR data of the 12-deoxy-16-hydroxyphorbol moiety (CD_2Cl_2, 500 MHz) δ 7.56 (1H, m, H-1), 5.61 (1H, d, J = 5.6 Hz, H-7), 5.41 (1H, s, OH-9), 4.18 (1H, d, J = 11.5 Hz, H-16a), 4.01 (1H, dd, J = 5.9, 13.1 Hz, H-20a), 3.96 (1H, dd, J = 5.9, 13.1 Hz, H-20b), 3.77 (1H, d, J = 11.5 Hz, H-16b), 3.24 (1H, br s, H-10), 3.08 (1H, br t, J = 4.8 Hz, H-8), 2.48 (1H, d, J = 19.2 Hz, H-5a), 2.43 (1H, d, J = 19.2 Hz, H-5b), 2.23 (1H, s, OH-4), 2.11 (1H, overlapped, H-12a), 1.99 (1H, m, H-11), 1.75 (3H, dd, J = 1.2, 2.7 Hz, CH_3-19), 1.64 (1H, dd, J = 11.8, 14.9 Hz, H-12b), 1.57 (1H, t, J = 5.9 Hz, OH-20), 1.16 (3H, s, CH_3-17), 1.01 (1H, d, J = 5.4 Hz, H-14), 0.89 (3H, d, J = 5.8, CH_3-18); ^{13}C NMR data of the 12-deoxy-16-hydroxyphorbol moiety (CD_2Cl_2, 125 MHz, based on DEPT, HMQC, and HMBC experiments) δ 208.7 (C-3), 161.0 (C-1), 140.9 (C-6), 133.2 (C-2), 129.4 (C-7), 76.0 (C-9), 74.2 (C-4), 69.3 (C-16), 68.2 (C-20), 64.6 (C-13), 56.1 (C-10), 38.9 (C-5), 38.6 (C-8), 36.6 (C-11), 31.9 (C-12), 30.3 (C-14), 26.6 (C-15), 18.6 (C-18), 11.0 (C-17), 10.4 (C-19); HMBC correlations of the 12-deoxy-16-hydroxyphorbol moiety were consistent with those of **2**; ^{1}H and ^{13}C NMR data of the dicarboxylic acid moiety, see Tables 1 and 2; HMBC correlations of the dicarboxylic acid moiety (CD_2Cl_2) C-1′→H-2′a, 2′b, 3′; C-2′→H-3′; C-3′→H-2′a, 2′b; C-4′→H-2′a, 2′b, 6′; C-8′→ H-6′, 10′a, 10′b, 14′; C-9′→H-8′, 14′, 16′; C-10′→H-8′, 15′; C-11′→H-12′, 13′, 16a; C-12′→H-14′; C-13′→H-14′, 15′; C-14′→H-10′b, 12′, 15′, 16′; C-15′→H-10′a, 13′, 14′, 16′; C-16′→H-10′a, 14′, 15′; C-20′→H-22′; C-21′→H-22′, 23′; C-22′→H-21′, 23′, 24′; C-23′→H-22′, 24′; C-24′→H-22′, 23′; ESIMS *m/z* 733 $[M + Na]^+$; HR-MALDI-TOF MS *m/z* 733.3559 $[M + Na]^+$ (calcd for $C_{44}H_{54}O_8Na$, 733.3711).

Acknowledgment. This work was financed by the Austrian Government within the scope of a developmental aid project organized by Sucher & Holzer, Graz, Austria. The authors also wish to thank Dr. F. Andreae (PiChem R&D, Graz, Austria) for recording the MALDI-TOF mass spectra, and Dr. O. Hofer (University Vienna) for performing the optical rotation measurements.

References and Notes

(1) Heller, J. *Physic Nut, Jatropha curcas L.: Promoting the Conservation and Use of Underutilized and Neglected Crops*; International Plant Genetic Resources Institute (IPGRI): Rome, 1996; p 13.
(2) Openshaw, K. *Biomass Bioenergy* **2000**, *19*, 1−15.
(3) Makkar, H. P. S.; Becker, K.; Sporer, F.; Wink, M. *J. Agric. Food Chem.* **1997**, *45*, 3152−3157.
(4) Gübitz, G. M.; Mittelbach, M.; Trabi, M. *Bioresour. Technol.* **1999**, *67*, 73−82.
(5) Felke, J. *Landwirtsch. Vers. Stn.* **1913**, *82*, 427−463.
(6) Adam, S. E. I.; Magzoub, M. *Toxicology* **1975**, *4*, 347−354.
(7) Abdu-Aguye, I.; Sannusi, A.; Alafiya-Tayo, R. A.; Bhusnurmath, S. R. *Hum. Toxicol.* **1986**, *5*, 269−274.
(8) Mampane, K. J.; Joubert, P. H.; Hay, I. T. *Phytother. Res.* **1987**, *1*, 50−51.
(9) Liberalino, A. A.; Bambirra, E. A.; Moraes-Santos, T.; Vieira, E. C. *Arq. Biol. Tecnol.* **1988**, *31*, 539−550.
(10) Gandhi, V. M.; Cherian, K. M.; Mulky, M. J. *Food. Chem. Toxicol.* **1995**, *33*, 39−42.
(11) Becker, K.; Makkar, H. P. S. *Vet. Hum. Toxicol.* **1998**, *40*, 82−86.
(12) Makkar, H. P. S.; Aderibigbe, A. O.; Becker, K. *Food Chem.* **1998**, *62*, 207−215.
(13) Evans, F. J. In *Naturally Occurring Phorbol Esters*; Evans, F. J., Ed.; CRC Press: Boca Raton, FL, 1986; Chapter 1, pp 1−31.
(14) Evans, F. J.; Soper, C. J. *J. Nat. Prod.* **1978**, *41*, 193−233.
(15) Aitken, A. In *Naturally Occurring Phorbol Esters*; Evans, F. J., Ed.; CRC Press: Boca Raton, FL, 1986; Chapter 10, pp 271−288.
(16) Fürstenberger, G.; Berry, D. L.; Sorg, B.; Marks, F. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 7722−7726.
(17) Horiuchi, T.; Fujiki, H.; Hirota, M.; Suttajit, M.; Suganuma, M.; Yoshioka, A.; Wongchai, V.; Hecker, E.; Sugimura, T. *Jpn. J. Cancer Res.* **1987**, *78*, 223−226.
(18) Hirota, M.; Suttajit, M.; Suguri, H.; Endo, Y.; Shudo, K.; Wongchai, V.; Hecker, E.; Fujiki, H. *Cancer Res.* **1988**, *48*, 5800−5804.
(19) Liu, S. Y.; Sporer, F.; Wink, M.; Jourdane, J.; Henning, R.; Li, Y. L.; Ruppel, A. *Trop. Med. Int. Health* **1997**, *2*, 179−188.
(20) Rug, M.; Ruppel, A. *Trop. Med. Int. Health* **2000**, *6*, 423−430.
(21) Samuelsson, G.; Farah, M. H.; Claeson, P.; Hagos, M.; Thulin, M.; Hedberg, O.; Warfa, A. M.; Hassan, A. O.; Elmi, A. H.; Abduraham, A. D.; Elmi, A. S.; Abdi, Y. A.; Alin, M. H. *J. Ethnopharmacol.* **1992**, *37*, 47−70.
(22) Adolf, W.; Opferkuch, H. J.; Hecker, E. *Phytochemistry* **1984**, *23*, 129−132.
(23) Gläser, S. Ph.D. Thesis, University of Heidelberg, Heidelberg, Germany, 1991, p 100.
(24) Wink, M.; Koschmieder, C.; Sauerwein, M.; Sporer, F. In *Biofuels and Industrial Products from Jatropha curcas*; Gübitz, G. M., Mittelbach, M., Trabi, M., Eds.; Dbv: Graz, Austria, 1997; Chapter 4.1, pp 160−166.
(25) Wink, M.; Grimm, C.; Koschmieder, C.; Sporer, F.; Bergeot, O. *Chemoecology* **2000**, *10*, 179−184.
(26) Gschwendt, M.; Hecker, E. *Tetrahedron Lett.* **1970**, *11*, 567−570.
(27) Schmidt, R. J.; Evans, F. J. *Experientia* **1977**, *33*, 1197−1198.
(28) Gschwendt, M.; Hecker, E. *Tetrahedron Lett.* **1969**, *10*, 3509−3512.
(29) Friebolin, H. *Ein- und Zweidimensionale NMR−Spektroskopie*; VCH: Weinheim, Germany, 1992; pp 87, 88.
(30) Burkert, U.; Allinger, N. L. *Molecular Mechanics*; ACS Monograph 177; American Chemical Society: Washington, DC, 1982.
(31) The numbering of the dicarboxylic acid residue is based on its presumed biogenesis (see below) and does not correspond to the numbering used in the literature cited.[18]
(32) Bergquist, K.-E. Ph.D. Thesis, University of Lund, Lund, Sweden, 1993, pp 44, 70.
(33) Nonfon, M. Ph.D. Thesis, University of Heidelberg, Heidelberg, Germany, 1986, p 33.
(34) Bergquist, K.-E.; Obianwu, H.; Wickberg, B. *J. Chem. Soc., Chem. Commun.* **1989**, 183−184.

NP020060D