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New 19-Acetoxyingol Diterpenes from the Latex of *Euphorbia poisonii* (Euphorbiaceae)

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Abstract—The poisonous latex of *Euphorbia poisonii* gave bioactive 3,12-diacetyl-8-nicotinyl-7-phenylacetyl 19-acetoxyingol (**1**) together with its less active congeners, 3,12-diacetyl-7-phenylacetyl 19-acetoxyingol (**2**) and 3-acetyl-7-phenylacetyl 19-acetoxyingol (**3**). The chemical structures of **1–3** were determined by spectroscopic methods including HRMS, DEPT, HETCOR, COSY, NOESY, HMQC and HMBC. The absolute configurations of the hydroxylated carbons in **2** and **3** were provisionally suggested by the Mosher ester method. Bioactivity-guided isolation, structure determination and cytotoxic activity of the new ingols are described. Copyright © 1996 Elsevier Science Ltd

Introduction

Euphorbia poisonii Pax. is a succulent Euphorbiaceae found in the tropics. It is known as 'Tinya' (Hausa) in northern Nigeria. The latex of this plant is a toxic skin irritant that has been used by peasant farmers in Kano to control bird pests on millet gardens. Quite often, rural communities in Nigeria attribute homicide deaths to 'Tinya' poisoning. Several ester derivatives of the tigliane[1,2] daphnane,[3] and ingol[4,5] diterpenes have previously been isolated from the latex of this plant. The tigliane diterpene esters are toxic[6] and are well known as tumor promoters.[6,7] They mimic the function of diacyl glycerol, the endogenous activator[8] of protein kinase C. The daphnane diterpene[3] esters of *E. poisonii* are potent skin irritants.[9]

Ingols are macrocyclic derivatives of the lathyrane diterpenes. The naturally occurring esters of ingol lack the irritant and toxic properties of the daphnanes or tiglianes to which they are biogenetically related.[10] Ingols, however, have attracted a considerable interest as antineoplastic agents.[11] In our search for potential pesticides and antitumor agents from plant sources, we have reinvestigated the latex of *E. poisonii* for bioactive compounds using the brine shrimp lethality test (BST)[12,13] as a guide. From the latex of this plant, three new 19-acetoxyingol esters (**1–3**) have been isolated. Herein, we report the isolation and structural determinations of **1–3** as well as their stereochemistries and cytotoxic activities.

Results and Discussion

Compound **1** was obtained as a white microcrystalline solid, mp 198–199 °C and $[\alpha]_D^{23}$ −30° (*c* 0.52 in $CHCl_3$).

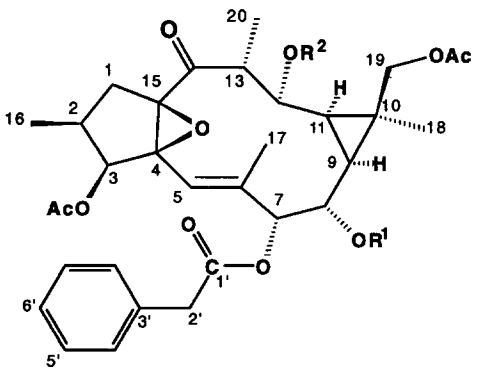
Key Words: *Euphorbia poisonii*, Euphorbiaceae, ingol diterpenes, cytotoxic activity.

High-resolution CIMS (calculated 732.3020, found 732.3042) suggested a molecular formula of $C_{40}H_{45}O_{12}N$, which was consistent with the carbon and hydrogen numbers counted in the NMR spectra. The IR of **1** lacked OH absorption signals, but exhibited bands characteristic of a ketone, an ester, and an aromatic ring. A close inspection of the UV spectrum of **1** in MeOH indicated the presence of an aromatic or a conjugated system. The NMR data (Table 1) revealed the presence of one ketone, five ester carbonyl carbons, one trisubstituted double bond, one nicotinyl, and one phenyl group. In addition, the spectral data showed signals for the following functionalities: one tertiary, one vinyl and two secondary methyl groups, three acetates, three methylenes, one of which is oxygen-substituted, eight methines, four of which are oxygen-substituted, one quaternary and two carbinyl carbons. The molecular formula of **1** corresponded to the presence of a total of 19 unsaturation equivalents. Thus, the remaining four unsaturation equivalents should be due to cyclic forms. These data together with the tracking of cross peaks in the ^1H-^1H COSY, HETCOR, HMQC, and HMBC spectra led to an ingol structure for **1**. The HMQC correlations revealed that the oxymethine carbons at δ 76.7, 76.2, 70.8, and 68.9 are part of the carbocyclic skeleton. Their proton signals at δ 5.17, 5.25, 4.94, and 4.57 are shifted downfield with respect to normal hydroxymethine protons suggesting the sites for ester linkages. The nicotinate and the phenylacetate groups were shown to be attached to C-8 and C-7 by HMBC experiments.[14] The nicotinate carbonyl resonance at δ 164.8 showed a three-bond connectivity to H-8 (δ 4.57) and H-7″ (δ 8.24). The phenylacetate carbonyl resonance (at δ 170.0) showed a two-bond connectivity to the benzylic methylene protons at δ 3.74 (H-2′) and a three-bond connectivity to δ 5.25 (H-7). Consequently, the remaining three acetates could only be attached to oxymethine carbons C-3, C-12, and oxymethylene

carbon C-19. For the protons at C-19 there were two distinct signals, corresponding to two diastereotopic and anisochronous protons. The multiplicity (doublet), coupling constant ($J=12$ Hz), and chemical shifts (δ 4.31 and 3.91) of the two protons are expected of an oxymethylene carbon attached to a quaternary carbon. The HMBC spectrum confirmed the attachment of C-19 to the quaternary carbon C-10 at δ 22.5. Taken together, these data suggested a 3,12-diacetyl-8-nicotinyl-7-phenylacetyl 19-acetoxyingol structure for **1** (Fig.1).

The structure and stereochemistry of ingol tetraacetate has been investigated by X-ray crystallographic analysis.[15,16] The C-8 acetoxy of ingol tetraacetate was previously assigned a beta configuration[15] and later corrected to alpha.[16] Molecular models of ingol esters revealed that the relative configurations at C-7, C-8, C-12, and C-13 could vary depending on the conformation of the cycloundecenone skeleton. The NOESY results and the coupling constants between H-7 and H-8 ($J=1.0$ Hz), H-8 and H-9 ($J=10$ Hz), H-11 and H-12 ($J=11$ Hz), H-12 and H-13 ($J=4.0$ Hz) were consistent with 13α-methyl, 12α-acetate, 8α-nicotinate, and 7α-phenylacetate orientations as previously described.[16] The multiplicity of H-7 and its coupling constant to H-8 (d, $J=1.0$ Hz) suggested a gauche relationship between the esters at C-7 and C-8. In analyzing the NOESY data, an α-configuration was assumed for H-9 and H-11 by analogy to other ingol-type[15–17] diterpenes of known absolute configuration.

For compound **2** $[\alpha]_D^{23}$ −4.3° (*c* 1.03, $CHCl_3$), the molecular formula $C_{34}H_{42}O_{11}$ (calcd for $C_{34}H_{42}O_{11}Na$ 649.2625, found 649.2625) was assigned on the basis of a molecular ion peak at *m/z* 627 (MH^+) in the FABMS and NMR measurements (Table 2). Analysis of the spectral data suggested that the structure of compound **2** was closely related to **1**. The major differences in the NMR spectra of **1** and **2** are in the ester and the oxymethine signals. The structure of **2** includes one hydroxymethine, one phenylacetate and three acetate groups. In addition, the ^1H NMR signal of H-8 was found to be shifted downfield in **1** with respect to **2** indicating the absence of an ester group at C-8.



1 R^1 = nicotinate, R^2 = Ac
2 R^1 = H, R^2 = Ac
2a R^1 = *S*-MTPA, R^2 = Ac
2b R^1 = *R*-MTPA, R^2 = Ac
3 $R^1 = R^2$ = H
3a $R^1 = R^2$ = *S*-MTPA
3b $R^1 = R^2$ = *R*-MTPA

Figure 1. Structures of **1–3**, **2a**, **2b**, **3a**, and **3b**.

The IR absorption at 3510 cm^{-1} and the CIMS base peak at *m/z* 609 ($M+H^+-H_2O$) confirmed the presence of an OH group in **2**. These data, in addition to full interpretation of the ^1H and ^{13}C NMR data (Table 2) and comparison with those of **1**, established a 19-acetoxyingol structure for **2**. The absolute configuration at C-8 and the attachment of a phenylacetate to C-7 were then studied using advanced Mosher ester methodology.[18]

This method analyzes the differences between the chemical shifts (Δ δH) of (*S*)- and (*R*)-MTPA ester derivatives of a chiral alcohol. The absolute value of Δ δH is proportional to the distance between a proton and the MTPA ester substituent. The assignment of the proton signals for (*S*)-MTPA ester **2a** and (*R*)-MTPA ester **2b** was assisted by ^1H-^1H COSY experiments and correlations with the ^1H NMR spectra of **2**. The $\Delta\ \delta H = \delta S - \delta R$ values for partial protons (Table 2) showed the absolute value of positive for H-9 and H-11 and negative for H-7 and H-17; thus, the absolute configuration at C-8 in **2** was determined to be *R*. The coupling constant values for H-7 and H-8 ($J=1.5$ Hz), H-8 and H-9 ($J=8.0$ Hz), H-11 and H-12 ($J=9.5$ Hz), H-12 and H-13 ($J=4.0$ Hz) and the stereochemical constraints of H-9 and H-11 established, essentially, the β-orientations for H-7, H-8, H-12, and H-13. These observations determined 7α-phenylacetoxyl, 8α-hydroxyl, 12α-acetoxyl, and 13α-methyl configurations for compound **2** (Fig. 1).

Compound **3** $[\alpha]_D^{23}+11°$ (*c* 0.93, $CHCl_3$) showed the molecular ion peak at *m/z* 585 ($M+H^+$) corresponding to a molecular formula of $C_{32}H_{40}O_{10}$. The basic structure of **3**, as suggested by NMR spectra, is similar to **2** and included one phenylacetate, two hydroxymethine, and two acetate groups. A diagnostic upfield shift in the ^1H NMR signals of H-8 and H-12 in **3** with respect to **1** indicated the absence of ester substituents at C-8 and C-12 (Table 1). The attachments of OH groups to C-8 and C-12 were, however, evident from the ^1H NMR data of the MTPA ester derivatives of **3** (Table 2). These data and the full interpretation of the COSY and ^{13}C NMR spectra (Table 1) established a 3-acetyl-7-phenylacetyl 19-acetoxyingol structure for **3**. The connectivities in the NOESY spectra of **2** and **3** were similar for H-7, H-8, H-19, and the cyclopentane protons (Table 1). In contrast to **2**, **3** showed a strong cross peak between H-5 and H-20 in the NOESY spectrum. The resonances of H-9 and H-11 at δ 1.19 and 1.22 in **2** were observed at δ 0.89 and 1.21 in **3** indicating differences in their stereochemical environments. Futhermore, the coupling values of H-7 to H-8 ($J=2.0$ Hz), H-8 to H-9 ($J=10.0$ Hz), H-11 to H-12 ($J=10.0$ Hz) and H-12 to H-13 ($J=3.0$ Hz) suggested

Table 1. ^{1}H and ^{13}C NMR spectral data of **1–3** in $CDCl_3$

No.	1 δH (*J*/Hz)	1 δC	No.	2 δH (*J*/Hz)	2 δC	No.	3 δH (*J*/Hz)	3 δC
1a	2.79 dd (15.0, 9.0)	31.5 CH_2	1a	2.76 dd (15.0, 9.0)	31.3 CH_2	1a	2.71 dd (15.0, 9.0)	31.0 CH_2
1b	1.69 d (15.0)		1b	1.68 d (15.0)		1b	1.71 d (15.0)	
2	2.53 sext (8.5)	29.5 CH	2	2.49 sext (8.5)	29.3 CH	2	2.47 sext (8.0)	29.5 CH
3	5.17 d (8.5)	76.7 CH	3	5.17 d (8.5)	76.5 CH	3	5.18 d (8.5)	76.4 CH
4		73.3 C	4		73.4 C	4		73.9 C
5	5.40 dq (1.5, 0.5)	117.1 CH	5	5.32 d (0.5)	116.5 CH	5	5.32 d (1.0)	116.8 CH
6		139.3 C	6		140.0 C	6		140.2 C
7	5.23 d (1.0)	76.2 CH	7	5.04 d (1.5)	79.3 CH	7	4.99 d (2.0)	79.8 CH
8	4.57 dd (10.0, 2.0)	68.9 CH	8	3.51 ddd (8.0, 5.0, 1.5)	69.4 CH	8	3.41 ddd (10.5, 5.5, 2.0)	68.5 CH
8-OH			8-OH	2.35 d (5.0)		8-OH	2.31 d (5.0)	
9	1.37 dd (11.0, 6.0)	31.0 CH	9	1.22 dd (10.5, 5.5)	28.5 CH	9	1.21 dd (7.5, obsc)	35.1 CH
10		22.5 C	10		21.7 C	10		21.4 C
11	1.34 dd (11.0, 6.0)	24.8 CH	11	1.19 dd (9.5, 5.5)	31.4 CH	11	0.89 dd (11.0, 9.0)	29.1 CH
12	4.93 dd (10.5, 4.0)	70.7 CH	12	4.87 dd (10.0, 4.0)	68.5 CH	12	3.23 ddd (10.0, 9.5, 3.0)	70.5 CH
12-OH							2.77 d (9.5)	
13	2.87 dq (7.5, 3.5)	43.4 CH	13	2.86 dq (7.5, 4.0)	43.0 CH	13	2.67 dq (7.5, 3.0)	42.9 CH
14		207.1 C	14		207.4 C	14		212.1 C
15		71.1 C	15		70.9 C	15		70.8 C
16	0.93 d (7.5)	16.9 CH_3	16	0.94 d (7.5)	16.9 CH_3	16	0.96 d (7.5)	16.6 CH_3
17	2.09 d (1.0)	17.4 CH_3	17	2.02 d (1.0)	17.7 CH_3	17	2.02 d (1.5)	17.8 CH_3
18	1.24 s	24.5 CH_3	18	1.09 s	24.7 CH_3	18	1.18 s	25.2 CH_3
19a	4.31 d (12.0)	65.5 CH_2	19a	4.18 d (12.0)	65.8 CH_2	19a	4.42 d (12)	66.0 CH_2
19b	3.91 d (12.0)		19b	4.05 d (12.0)		19b	4.13 d (12.5)	
20	1.03 d (7.5)	13.2 CH_3	20	1.02 d (7.0)	13.3 CH_3	20	1.21 d (7.5)	14.5 CH_3
1′		170.0 C	1′		170.4 C	1′		170.5 C
2′	3.74 s	41.5 CH_2	2′	3.75 s	41.3 CH_2	2′	3.75 s	41.3 CH_2
3′		133.7 C	3′		133.8 C	3′		133.9 C
4′ and 8′	7.18–7.31 m	129.2 CH	4′ and 8′		129.3 CH	4′ and 8′		129.4 CH
5′ and 7′	7.32–7.36 m	127.3 CH	5′ and 7′	7.31 m	128 6 CH	5′ and 7′	7.31 m	128.6 CH
6′	7.18–7.31 m	128.6 CH	6′		127.2 CH	6′		127.2 CH
1″		164.8 C	3-Ac	2.12 s	21.0 CH_3	3-Ac	2.09 s	20.9 CH_3
2″		126.2 C			170.6 C			170.8 C
3″	9.15 dd (2.0, 1.0)	153.4 CH	12-Ac	2.05 s	20.8 CH_3	19-Ac	2.04 s	20.5 CH_3
5″	8.77 dd (4.5, 2.0)	150.5 CH			170.5 C			170.6 C
6″	7.40 ddd (8.0, 4.5, 1.0)	123.3 CH	19-Ac	2.04 s	20.5 CH_3			
7″	8.24 dt (8.0, 2.0)	136.9 CH			170.6 C			
3-Ac	2.08 s	20.9 CH_3						
12-Ac	1.98 s	20.7 CH_3						
19-Ac	1.78 s	20.5 CH_3						

Table 2. Partial ^{1}H NMR spectral data for **2a**, **2b**, **3a**, and **3b** in $CDCl_3$

No.	2a (*S*-MTPA)	2b (*R*-MTPA)	Δδ	3a (*S*-MTPA)	3b (*R*-MTPA)	Δδ
17	2.07 d (1.0)	2.11 d (2.0)	−0.04	2.01 d (1.5)	2.04 d (1.5)	−0.03
7	5.15 s	5.19 s	−0.04	4.95 d (1.5)	5.14 d (1.5)	−0.19
8	4.79 dd (8.5, 2.0)	4.75 dd (7.0, 5.5)	*R*	4.36 dd (11)	4.66 dd (11)	*R*
9	1.32 dd (10.5, 8.5)	1.28 dd (9.5, 6.0)	+0.05	1.13 dd (7.0, 3.5)	1.01 dd (7.0, 3.5)	+0.12
11	1.29 dd (10.5, 8.5)	1.24 dd (9.5, 6.0)	+0.05	0.90 dd (7.0, 4.5)	0.88 dd (7.5, 5.5)	+0.02
12				3.60 d (2.5)	3.64 d (2.5)	*R*
13				2.63 dq (7.5, 3.0)	2.69 dq (7.5, 3.0)	−0.06
16				0.96 d (7.5)	0.98 d (7.5)	−0.02
17				2.01 d (1.5)	2.04 d (1.5)	−0.03

the β- orientations for H-7, H-8, H-12, and H-13. This set of data determined 7α-phenylacetoxyl, 8α-hydroxyl, 12α-hydroxyl and 13α-methyl orientations for **3** (Fig. 1). The differences between the Δ δH values of the (*S*)-MTPA ester (**3a**) and the (*R*)-MTPA ester (**3b**, Table 2) provisionally established the C-8 (*R*) and C-12 (*R*) configurations.

Compounds **1–3** were evaluated for cytotoxicities in a panel of six human solid tumor cell lines using adriamycin as a positive control. Compound **1** was modestly cytotoxic, exhibiting nonselective ED_{50} values ranging from 2 to 4 μg/mL with the specific ED_{50} (μg/mL) values observed as follows: lung carcinoma (A-549)[19] 2.2, breast carcinoma (MCF-7)[20] 3.7, colon adrenocarcinoma (HT)[21] 3.0, kidney carcinoma (A-498)[19] 2.1, prostrate adenocarcinoma (PC-3)[22] 2.0, and pancreatic carcinoma (PACA-2)[23] 2.31. Compounds **2** and **3** were not significantly active in the six cell lines tested. The most potent activity observed for **2** was against the kidney carcinoma (A-498), ED_{50} 25 (μg/mL). Compound **3** was weakly but selectively active at ED_{50} 15 μg/mL against the prostate adrenocarcinoma (PC-3). In the same run, adriamycin gave ED_{50} values of 4.2×10^{-3}, 3.8×10^{-1}, 3.9×10^{-2}, 1.9×10^{-3}, 6.4×10^{-2}, and 1.6×10^{-2} μg/mL.

Experimental

Instrumentation

Mps: uncorrected, on a Fisher–Johns apparatus; UV: MeOH, on a Beckman DU-7 spectrophotometer; IR: film, on a Perkin–Elmer 1600 FTIR spectrophotometer; Optical rotations on a Perkin–Elmer 241 polarimeter; ^1H and ^{13}C NMR: on a Varian VXR-500S spectrometer (^1H at 500 MHz, ^{13}C at 125.75 MHz) for solutions in $CDCl_3$ with TMS as internal reference; Low-resolution CIMS: Finnigan 4000 spectrometer; Low- and high-resolution FABMS: Kratos MS 50 through peak matching on dithiothreitol–dithioerythritol matrix with NaI addition. Open-column liquid chromatography was performed over silica (Merck, 60–200 mesh). HPLC was run on a Rainin system equipped with Dynamax software (Rainin Instrument Company, Inc.), two Rainin HPXL pumps, a Dynamax UV-1 variable wavelength detector set at 248 nm, and a normal phase Dynamax-60 A 8 μm silica gel column (21 × 250 mm i.d.). Analytical TLC was performed on pre-coated Merck glass sheets (Whatman K6F Silica gel 60°A) and viewed under a UV lamp or sprayed with 5% phosphomolybdic acid in EtOH followed by heating. (*R*)- and (*S*)-2-methoxy-2-(trifluoromethyl)-phenyl acetyl chloride are Aldrich products. (*R*)- and (*S*)-2-methoxy-2-(trifluoromethyl)phenyl acetic acid (MTPA) esters were prepared by a standard procedure.[18]

Plant material

The latex of *E. poisonii* Pax. was collected into an equal volume of absolute EtOH from plants found in the Kombosto local government area of Kano state, Nigeria, in May 1994 and stored in a freezer. The botanical identification of the plant was confirmed by Mr Ali Garko and Dr Y. Karatella of the Department of Biological Sciences, Bayero University, Kano. A reference specimen of the plant is being cultivated in the Botanical Garden of Bayero University, Kano.

Bioassays

Extracts, partition fractions, bulked chromatographic eluents, and pure compounds were tested for lethality against brine shrimp larvae (*Artemia salina* Leach) (BST).[12,13] Cytotoxicities to human tumor cells were carried out at the Purdue Cancer Center, using a 7-day MTT assay and standard protocols for A-549 (lung carcinoma),[19] MCF-7 (human breast carcinoma),[20] HT-29 (human colon adrenocarcinoma),[21] A-498 (kidney carcinoma),[19] PC-3 (prostate adenocarcinoma)[22] and PACA-2 (pancreatic carcinoma)[23] with adriamycin as a positive control.

Extraction and isolation

The ethanol-preserved latex of *E. poisonii* was concentrated under vacuum to yield a combined extract (706 g) that was partitioned between H_2O (2.0 L) and CH_2Cl_2 (2 × 2.0 L). The CH_2Cl_2 soluble fraction was evaporated to dryness to give a residue (214 g; BST $LC_{50}>1000$ μg/mL), which was further partitioned between 10% aq MeOH (1.6 L) and hexane (2 × 1.6 L). Upon evaporation of the solvent, the hexane soluble fraction (138 g; BST $LC_{50}>1000$ μg/mL) and the MeOH soluble fraction (33 g, BST LC_{50} 114 μg/mL) gave solid residues. The MeOH soluble residue exhibited significant cytotoxic activities against the human tumor cells (A-549, ED_{50} 4.8 μg/mL; MCF-7, ED_{50} 27 μg/mL; HT-29, ED_{50} 27 μg/mL; PC-3, ED_{50} 12 μg/mL; and PACA-2, ED_{50} 23 μg/mL; adriamycin gave respective ED_{50} values of 3.9×10^{-3}, 1.51×10^{-1}, 3.4×10^{-2}, 3.2×10^{-2}, and 2.5×10^{-3} μg/mL for the same run). A portion of the MeOH soluble residue (26 g) was chromatographed over Si gel (250 g) on an open column (60 mm i.d.), using a hexane:EtOAc gradient mixtures as eluent. The eluents were analyzed on TLC and bulked into 12 fractions. Bioactive fraction EP-08 (1.17 g; BST LC_{50} 0.5 μg/mL), eluted with hexane:EtOAc (8:2), was subjected to a second open column (20 mm i.d. chromatography over Si gel (35 g), eluting with hexane:CH_2Cl_2 and CH_2Cl_2:EtOAc gradients. Cytotoxic fractions EPO8–16 and EPO8–17 eluted with CH_2Cl_2:EtOAc (9:1) were subjected to a repetitive normal phase gradient purification on HPLC using 1–8% (MeOH:THF, 9:1) in hexane (10 mL/min) to yield, respectively, ingols **2** (29.0 mg), **3** (9.5 mg), and **1** (23.3 mg).

3,12-Diacetyl-8-nicotinyl-7-phenylacetyl 19-acetoxyingol (1). White microcrystalline solids, mp 198–199 °C; $[\alpha]_D^{23}$ −30° (*c* 0.52, $CHCl_3$); UV λ_{max} (MeOH) nm (log ε): 263 (3.48), 247 (3.41) 201 (4.37); IR ν_{max} (film) cm^{-1}: 2966, 1738, 1614, 1536, 1095, 992. ^1H and ^{13}C NMR: see Table 1; CIMS: *m/z* (%) 732 ($M+H^+$; 48),

672 ($M+H^+$-CH_3CO_2H; 100); HRCIMS: calcd for $C_{40}H_{46}O_{12}N$ 732.3042, found 732.3020.

3,12-Diacetyl-7-phenylacetyl 19-acetoxyingol (2). White flakes, mp 180–182 °C; $[\alpha]_D^{23}$ −4.3° (*c* 1.03, $CHCl_3$); UV λ_{max} (MeOH) nm (log ε): 202 (3.78), 201 (3.75); IR_{max} (film) cm^{-1}: 3510, 3003, 1732, 1535, 1496, 796. ^1H and ^{13}C NMR: see Table 2; CIMS: *m/z* (%) 609 (MH^+-H_2O; 14), 567 (7); 431 (5), 391 (7), 371 (17), 329 (10) 311 (14), 283 (11), 257 (8), 137(34), and 91 (100); LRFABMS: *m/z* 609 $(M+H)^+-H_20$ (100); HRFABMS: calcd for $C_{34}H_{42}O_{11}Na$ 649.2625 $(M+Na)^+$, found 649.2625.

3-Acetyl-7-phenylacetyl 19-acetoxyingol (3). $[\alpha]_D^{23}$ +11° (*c* 0.93, $CHCl_3$); UV λ_{max} (MeOH) nm (log ε): 201 (4.07); IR ν_{max} (film) cm^{-1}: 3526, 3119, 2934, 1737, 1538, 1454, 894, 787. ^1H and ^{13}C NMR: see Table 3; CIMS: *m/z* (%) 585 (1), 567 ($M^+ + H - H_2O$; 55), 525(41), 507 (15), 431 (16), 389 (25), 371 (47), 329 (22), 311 (29), and 137 (100).

***(R)*- and *(S)*-Mosher esters.** To a vial containing the ingol (2.2 mg) was added pyridine (2 drops), 4-(dimethylamino)pyridine (1 mg), CH_2Cl_2 (1 mL) and a large excess of (*R*)- or (*S*)-2-methoxy-2-(trifluoromethyl)phenyl acetyl chloride. The vial was capped and left at room temperature. The reaction was monitored by TLC, diluted after 2 days with hexane (1 mL) and passed through a disposable pipette containing Si gel. The column was eluted with 4 mL of each of the following solvents: hexane, CH_2Cl_2, and CH_2Cl_2:EtOAc mixtures. The Mosher esters **2a** and **2b** or **3a** and **3b** were eluted with a mixture of CH_2Cl_2 (4 mL) and EtOAc (8 or 16 drops). ^1H NMR: see Table 2.

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