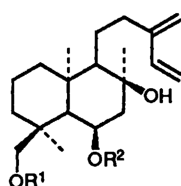
Biotransformation of *ent*-13-*epi*- and *ent*-Manoyl Oxides by *Rhizopus nigricans* Cultures

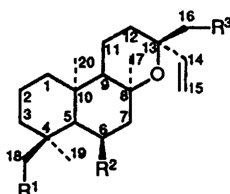
Andrés García-Granados,*[,a] Antonio Martínez,[a] Juan P. Martínez,[a] M. Esther Onorato,[b] and J. M. Arias[c]
[a] *Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain*
[b] *Análisis y Determinación de Estructuras, Servicios Técnicos, Universidad de Granada, 18071 Granada, Spain*
[c] *Departamento de Microbiología, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain*

Microbial transformation of *ent*-6α,18-diacetoxy-16-hydroxy-13-*epi*-manoyl oxide (3) has been carried out with *Rhizopus nigricans* cultures, which converted the substrate (3) into its 20-hydroxy-, *ent*-3β-hydroxy-, and (14*S*)-spiro derivatives. *R. nigricans* transformed *ent*-6α,18-diacetoxy-16-hydroxymanoyl oxide (5) into its *ent*-11β-hydroxy and (14*R*)-spiro derivatives. The metabolite structures have been determined spectroscopically and sometimes by chemical correlation as well.

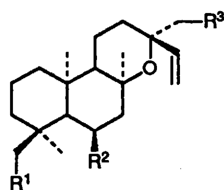
As described, highly functionalized manoyl oxides have proved to be useful as antihypertensive agents[1] and in the treatment of respiratory constrictions,[2] glaucoma,[3] skin complaints,[4] and congestive heart failure.[5] Some of these oxides are obtainable by microbial transformation.[6–8] We are thus performing a wide series of biotransformations of differently functionalized *ent*-13-*epi*-manoyl oxides[9,10] to obtain *ent*-13-*epi*-manoyl oxides highly functionalized for biological testing. As we described in a previous paper, the *ent*-8α-hydroxylabda-13(16),14-diene system can be cyclized to *ent*-16-hydroxymanoyl oxides *via*



	R^1	R^2
(1)	H	H
(2)	Ac	Ac



	R^1	R^2	R^3
(3)	OAc	OAc	OH
(4)	H	H	H
(15)	OAc	OAc	OAc



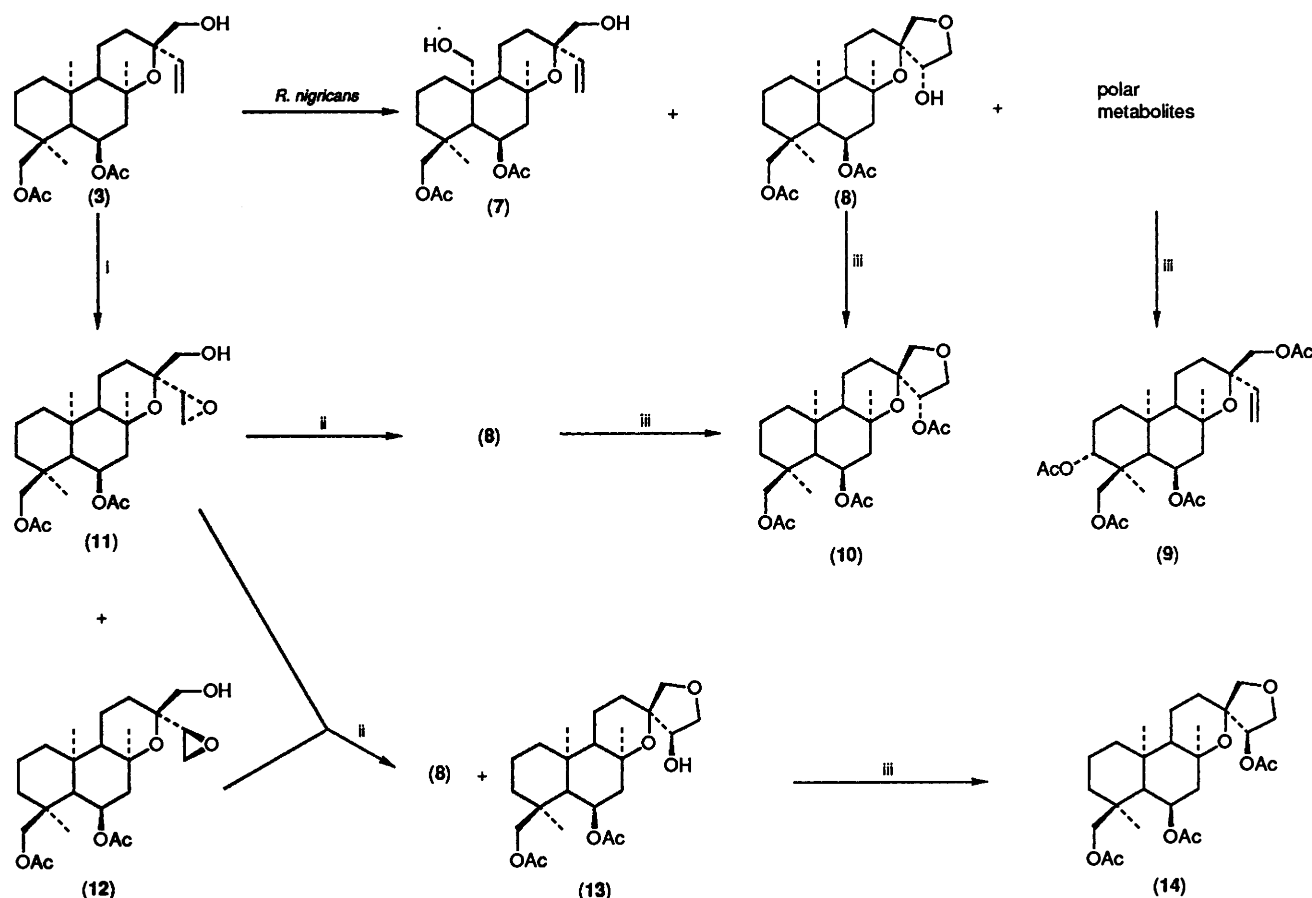
	R^1	R^2	R^3
(5)	OAc	OAc	OH
(6)	H	H	H

epoxidation.[9] Therefore, we have acetylated an abundant natural diterpene isolated from some Sideritis[11,12] [*ent*-6α,8α,18-trihydroxylabda-13(16),14-diene, andalusol[13] (1)] to give the diacetate[13] (2), which was then epoxidized to give substrates (3) [with an *ent*-13-*epi*-manoyl oxide skeleton (4)] and (5) [with an *ent*-manoyl oxide skeleton (6)]. These were then biotransformed using *Rhizopus nigricans*, as described below.

Results and Discussion

Biotransformation of substrate (3) (1 g) with *R. nigricans* for 6 days gave metabolites (7) (264 mg, 25%), (8) (60 mg, 6%), and a mixture of more polar compounds from which the tetra-acetate (9) (25 mg, 2%) was isolated after acetylation (Scheme 1). Substrate (3) (262 mg, 26%) was also recovered unaltered. The main metabolite (7) was shown to have a new primary hydroxy group, which was detected by ^{1}H and ^{13}C NMR spectroscopy (see Experimental section and Table 1). This functionalization could be similar to that found previously for a 20-hydroxy derivative.[9] Thus, the ^{13}C NMR data for metabolite (7) were in accordance with previous data.[9] We have now performed a series of 1D- and 2D-NMR experiments to assign the ^{1}H and ^{13}C signals unequivocally and made several NOE-difference experiments which are explained in the Figure. We have proved in this way that the new hydroxy group is at C-20.

Metabolite (8) had a molecular weight of 438 [439 $(M + 1)^+$, MS (CI)], indicating that a new oxygen atom has been introduced into the substrate (3) (*M* 422). Metabolite (8) did not have the original double bond (^{1}H and ^{13}C NMR). ^{13}C NMR data of metabolite (8) indicated a different oxygenated methylene carbon (δ 79.77) and similar values for the carbons in rings A and B, as well as at C-11 and C-12. The chemical shifts of C-13 for substrate (3) and metabolite (8) were considerably different (δ 76.29 and 82.88 respectively). Moreover, metabolite (8) showed two new oxygenated carbons [δ 76.11 (methyne) and 75.14 (methylene)]. Metabolite (8) was not a 16,17-glycol (thin layer chromatography polarity; non-formation of isopropylidenedioxy derivative). Acetylation of metabolite (8) led to a triacetate (10). Metabolite (8) must hence be a spiran tetrahydrofuran derivative hydroxylated at C-14. Metabolite (8) may result from the epoxidation of the double bond of the substrate (3) and a subsequent intramolecular cyclization with the participation of the hydroxy group at C-16. A series of NOE-difference experiments could not conclusively determine the configuration at C-14. For this purpose we epoxidized substrate (3) with MCPBA to give a mixture of two epoxy compounds (11) and (12), from which the epoxide (11) was separated with difficulty. Treatment of the epoxide (11) with Al_2O_3 (see Experimental section) gave a compound identical



Scheme 1. Biotransformation of substrate (3) with *R. nigricans* for 6 days and chemical correlation of metabolite (8). *Reagents:* i, MCPBA; ii, Al_2O_3; iii, Ac_2O/Py.

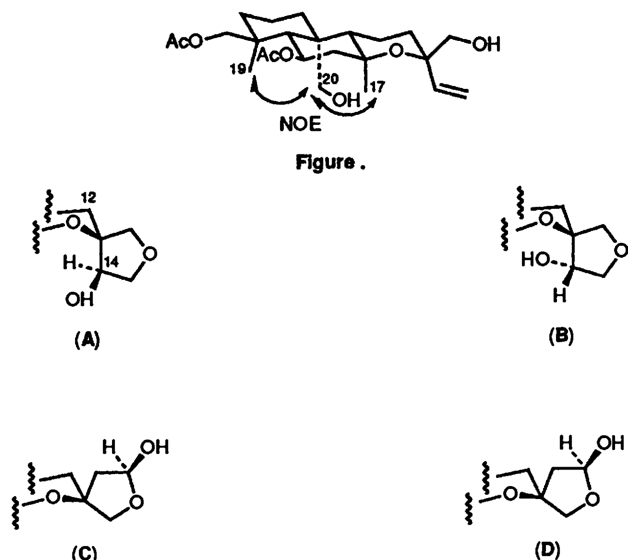


Figure.

with metabolite (**8**). The acetate of this product was also identical with the triacetate (**10**). Treatment of the mixture of epoxides (**11**) and (**12**) with Al_2O_3 gave a mixture of compounds (**8**) and (**13**) which were then separated. A comparison of the ^1H and ^{13}C NMR spectra of both C-14 epimers will now permit the configuration at this carbon to be deduced. Thus, once the assignments of the complex signals in the ^1H NMR spectra of metabolite (**8**) and of its epimer (**13**), with the aid of COSY and C/H correlation experiments were complete, the consideration of the chemical shifts of 14-H and C-12 were decisive in assigning the configuration at C-14 of both epimeric compounds. As can be seen in partial structures (**A**) and (**B**), the proton at C-14 in (**B**) must be more deshielded than that in (**A**), because of its proximity to the oxygen of the 8,13-epoxy system, and the C-12 in (**B**) must be more shielded than that in (**A**) because the γ-*syn* effect produced on C-12 by the hydroxy group at C-14. The ^1H and ^{13}C NMR chemical shifts of 14-H and C-12 of metabolite (**8**), its epimer (**13**) and the corresponding acetate derivatives (**10**) and (**14**) are indicated in Table 2, which clearly indicates that metabolite (**8**) may have a partial structure (**B**) and hence its epimer (**13**) had partial structure (**A**). Thus *R. nigricans* epoxidized substrate (**3**) at the *Si* face of the double bond to give an epoxide with posterior cyclization to metabolite (**8**), resulting in the structure (13*S*,14*S*)-*ent*-6α,18-diacetoxy-14-hydroxy-8α,13; 15,16-diepoxylabdane.

The tetra-acetate (**9**) was isolated after acetylation of the mixture of polar metabolites obtained by the incubation of substrate (**3**) with *R. nigricans* (see Scheme 1). Its MS, IR, and ^1H NMR spectra indicated four acetoxy groups. In addition to the expected signal for protons at C-6, C-16, and C-18, another signal for an axial proton, geminal to an acetoxy group, appeared, only coupled to two vicinal protons. The ^{13}C NMR spectrum (see Table 1) confirmed that this new acetoxy group was situated at C-3 of substrate (**3**), by comparison with the chemical shifts of *ent*-6α,16,18-triacetoxy-13-*epi*-manoyl oxide[12] (**15**), and taking into consideration the effects observed for such types of function with similar compounds.[9] The mixture described above thus derived from the migration of the acetoxy group in between the hydroxy groups at C-3 and C-18.[9]

The biotransformation of substrate (**5**) [epimer at C-13 of the

Table 1. ^{13}C NMR chemical shifts (δ) of compounds (3), (5), (7), (8), (9), (13), (16), (17), and (21).

C	(3)	(5)	(7)	(8)	(9)	(13)	(16)	(17)	(21)
1	38.45	38.23	32.79	38.58	36.65	38.53	38.45	38.54	38.56
2	17.36	17.29	17.44	17.48	22.66	17.36	17.25	17.48	17.46
3	36.84	36.81	36.78	36.98	74.28	36.93	36.86	37.00	36.98
4	36.33	36.41	36.10	36.48	40.12	36.42	36.44	36.47	36.48
5	52.08	51.90	52.64	52.29	50.39	52.25	53.84	52.23	52.19
6	70.25	70.53	69.34	70.48	69.66	70.17	70.79	70.46	70.30
7	49.00	49.78	49.48	49.33	49.34	49.20	50.62	48.68	48.39
8	75.34[a]	76.68	75.49[a]	74.82	75.07[a]	75.35	76.21	74.72	75.31
9	57.79	52.61	58.35	56.84	57.63	55.39	64.01	57.37	57.46
10	37.50	37.86	43.03	37.76	37.54	37.86	38.66	37.70	37.68
11	15.39	14.67	18.75	16.20	15.52	15.39	55.08	15.57	15.81
12	28.12	26.42	29.75	26.35	29.75	31.04	38.29	26.90	33.03
13	76.29[a]	76.68	76.76[a]	82.88	74.72[a]	79.15	74.45	80.88	78.77
14	143.53	143.61	143.22	76.11	142.96	74.39	143.17	78.75	78.60
15	113.67	114.31	114.17	75.14	114.01	74.39	143.17	70.46	72.09
16	69.49	68.72	69.69	79.77	67.10	77.09	68.67	75.11	73.16
17	24.97	27.05	24.53	25.44	25.12	24.87	28.57	24.20	23.85
18	74.07	74.36	74.04	74.26	71.32	74.17	74.45	74.27	74.18
19	17.64	17.83	18.50	17.82	13.18	17.85	17.87	17.82	17.82
20	17.31	16.41	62,21	17.22	17.28	16.88	18.66	17.27	17.30
CO*Me*	21.61	21.70	21.69	21.72	21.64	21.68	21.72	21.74	21.74
	21.02	21.07	21.16	21.12	21.26	21.09	21.06	21.14	21.13
					21.10				
*CO*Me	170.16	171.24	171.24	171.29	171.14	171.20	171.27	171.29	171.24
	171.16	170.26	170.21	170.37	170.88	170.28	170.28	170.23	170.31
					170.67				
					170.33				

[a] These assignments may be interchanged.

Table 2. NMR chemical shifts (δ) of 14-H and C-12 for compounds (8), (10), (13), (14), (17), (18), (21), and (22).

Compd.	14-H	C-12
(8)	4.31	26.35
(10)	5.45	26.55
(13)	3.79	31.04
(14)	4.95	33.52
(17)	4.09	26.90
(18)	4.96	27.07
(21)	3.72	33.03
(22)	4.98	33.16

These assignments were made with the aid of 2D-NMR C/H correlation

incubated substrate (3)] with *R. nigricans* for 5 days gave unaltered substrate (5) (230 mg, 25%), metabolite (16) (215 mg, 23%), and metabolite (17) (80 mg, 9%) (Scheme 2). Metabolite (16) showed a new secondary hydroxy group (MS, ^1H and ^{13}C NMR) compared to substrate (6). The proton geminal to the new hydroxy group produced a signal at δ 4.41 (^{1} H, ddd, J_1 = J_2 7.0 and J_3 4.7 Hz) in the ^1H NMR spectrum. Moreover, this hydroxy group produced notable deshielding, principally in the methyl groups at C-17 and C-20 (δ +0.29 and +0.37, respectively) as well as considerable modifications of the signals of the AB system of protons at C-16 (see Experimental section). ^1H and ^{13}C NMR data for the metabolite (16) (see Table 1) allowed us to assign an *ent*-11β disposition to its new hydroxy group.

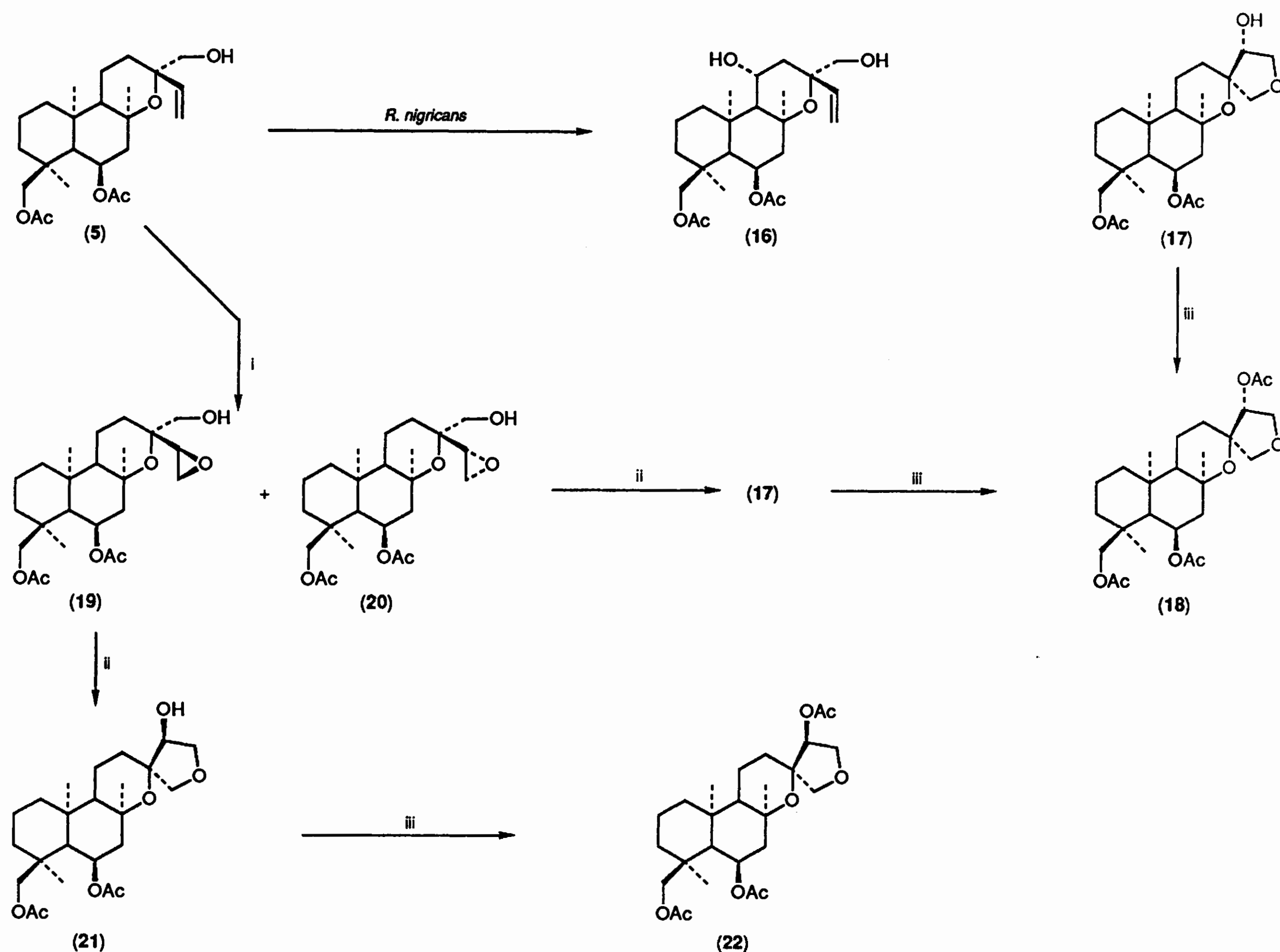
The metabolite (17) showed to some extent similar spectral data to the previously described metabolite (8) and its epimer (13). Thus, the metabolite (17) had one oxygen atom more than substrate (5) and its acetylation gave a triacetate (18). Once more there arose the problem of the determination of configuration at C-14 and again the NOE-difference experiments made on the acetate (18) were inconclusive. Consideration of the ^1H and ^{13}C NMR chemical shifts of one epimer alone is, in our opinion, insufficient for deciding this configuration at C-14. Hence, we epoxidized substrate (5) with MCPBA to give two epoxy compounds (19) (75 mg, 36%) and (20) (63 mg, 30%), the separation of which was feasible on this occasion. The cyclization on Al_2O_3 of the epoxide (19) gave the spirane (21) and that of the epoxide (20) gave the metabolite (17). The configuration at C-14 of epoxides could only be determined after the configuration at C-14 of its cyclized derivatives had been worked out.

Now, of the two possible partial structures (C) and (D) for spiranes (17) and (21), partial structure (D) could give rise to a more deshielded proton at C-14 and a more shielded one at C-12 (by the γ-*syn* effect produced by the hydroxy group at C-14). The 14-H and C-12 chemical shifts of the metabolite (17), the spirane (21), and its triacetates (18) and (22) are indicated in Table 2. As can be seen, the metabolite (17) had the partial structure (D) and hence the structure (13*R*, 14*R*)-*ent*-6α,18-diacetoxy-14-hydroxy-8α,13;15,16-di-epoxylabdane.

In conclusion, the substrate configuration at C-13 influences the behaviour of *R. nigricans*. Thus, for the substrate (3) (configuration 13-*epi*), the fungus mainly produced hydroxylation at C-20, and C-3 to a lesser extent, which agrees with our observations in a previous study.[9] Here it also epoxidized the vinylic system selectively at the *Si* face. In the substrate with the configuration 13-*normal*, *R. nigricans* mainly hydroxylated axially at C-11 and, to a lesser degree epoxidized the vinylic system, also selectively, at *Re*-face.

Experimental

Isolation of Starting Material (1).—*ent*-6α,8α,18-Trihydroxyl-abda-13(16),14-diene (andalusol; 1)[13] used in this work was isolated from *Sideritis varoi*[11] and *Sideritis arborescens*.[12]



Scheme 2. Biotransformation of substrate (5) with *R. nigricans* for 5 days and chemical correlation of metabolite (17). *Reagents:* i, MCPBA; ii, Al_2O_3; iii, Ac_2O/Py.

Acetylation of Andalusol (1).—Andalusol (1) (5 g) was acetylated with pyridine–Ac_2O (40:20 ml) for 12 h at room temperature. Chromatography on a silica gel column yielded *ent*-6α,18-diacetoxy-8α-hydroxylabda-13(16),14-diene[13] (2) (4.5 g, 71%).

Cyclization of the Diacetate (2).—The diacetate (2) (4 g) was dissolved in $CHCl_3$ (100 ml) and MCPBA (2.5 g) was added. After 24 h at room temperature the mixture was chromatographed on a silica gel column to give ent-6α,18-*diacetoxy*-16-*hydroxy*-13-epi-*manoyl oxide* (3) (1.32 g, 32%) as an oil: $[\alpha]_D$ −59° (*c* 1 in $CHCl_3$); ν_{max} 3 450 (OH), 3 090 (C=C), and 1 745 and 1 260 cm^{-1} (OCO); δ_H 0.77 and 0.78 (3 H each, s, 4-Me and 10-Me), 1.29 (3 H, s, 8-Me), 1.94 and 2.02 (3 H, each, s, AcO groups), 2.92 and 3.23 (2 H, AB system, *J* 10.9 Hz, 16-2 H), 3.54 and 3.96 (2 H, AB system, *J* 10.8 Hz, 18-2 H), 4.98 (1 H, ddd, $J_1 = J_2$ 11.3 Hz and J_3 3.8 Hz, 6-H), 5.00–5.06 (2 H, part AB of a ABX system, 15-2 H), and 5.81 (1 H, part X of an ABX system, $J_{AX} + J_{BX}$ 29 Hz, 14-H); δ_C see Table 1; *m/z* (CI) 423 ($[M + 1]^+$ 1%), 363 ($[M + 1]^+$ − AcOH, 100) (Found: C, 68.1; H, 9.2. $C_{24}H_{38}O_6$ requires C, 68.22, H, 9.06%); and ent-6α,18-*diacetoxy*-16-*hydroxymanoyl oxide* (5) (1 g, 24%) also as an oil: $[\alpha]_D$ −61° (*c* 1 in $CHCl_3$); ν_{max} 3 504 (OH), 3 085 (C=C), and 1 736 and 1 238 cm^{-1} (OCO); δ_H 0.82 and 0.89 (3 H each, s, 4-Me and 10-Me), 1.34 (3 H, s, 8-Me), 1.99 and 2.05 (3 H, each, s, AcO groups), 3.24 (2 H, collap. AB system, 16-2 H), 3.66 and 3.99 (2 H, AB system, *J* 10.8 Hz, 18-2 H), 5.02 (1 H, ddd, $J_1 = J_2$ 11.3 and J_3 3.9 Hz, 6-H), 5.10–5.26 (2 H, part AB of an ABX system, 15-2 H) and 5.79 (1 H, part X of an ABX system, $J_{AX} + J_{BX}$ 28.2 Hz, 14-H); δ_C see Table 1; *m/z* (CI) 423 ($[M + 1]^+$, 1%), 363 ($[M + 1]^+$ − AcOH, 58) (Found: C, 68.4; H, 9.2. $C_{24}H_{38}O_6$ requires C, 68.22; H, 9.06%).

Organism, Media, and Cultures Conditions.—*Rhizopus nigricans* CECT 2672 (ATCC 10404) from Colección Española de Cultivos Tipo[14] was used in these studies. Medium YEPGA containing yeast extract (1%), peptone (1%), glucose (2%), agar (2%) in water at pH 5 was used for storage of *R. nigricans*. A medium of the following composition was used in all transformation experiments: peptone (0.1%), yeast extract (0.1%), beef extract (0.1%), and glucose (0.1%) at pH 5.7 in water. Erlenmeyer flasks (250 ml) containing 60 ml of medium were inoculated with a dense suspension of *R. nigricans*. Incubation were maintained at 28 °C with orbital stirring (150 rpm) for 6 days, after which substrates (3) and (5) in EtOH were added.

Biotransformation of Substrate (3).—Substrate (3) (1 g) was dissolved in EtOH (20 ml) and the solution distributed among 20 Erlenmeyer flask cultures. The latter were incubated for 6 days, after which they were filtered and pooled; the cells were washed with water and the liquid was saturated with NaCl and extracted with CH_2Cl_2. The two extracts were mixed, dried ($MgSO_4$), and evaporated at 40 °C under reduced pressure to give a mixture of compounds (800 mg). This mixture was

chromatographed on a silica gel column to give starting product (**3**) (262 mg, 26%), ent-6α,18-*diacetoxy*-16,20-*dihydroxy*-13-epi-*manoyl oxide* (**7**) (264 mg, 25%) as an oil: $[\alpha]_D$ −51° (*c* 1 in $CHCl_3$); ν_{max} 3 471 (OH), 3 080 (C=C), and 1 736 and 1 245 cm^{-1} (OCO); δ_H 0.76 (3 H, s, 4-Me), 1.49 (3 H, s, 8-Me), 2.09 and 2.00 (3 H each, s, AcO groups), 2.97 and 3.28 (2 H, AB system, *J* 11.0 Hz, 16-2 H), 3.76 and 3.95 (2 H, AB system, *J* 11.0 Hz, 20-2 H), 3.65 and 3.99 (2 H, AB system, *J* 10.9 Hz, 18-2H), 5.00–5.16 (3 H, part AB of an ABX system, 16-2 H and signal of 6-H), and 5.87 (1 H, part X of an ABX system, $J_{AX} + J_{BX}$ 28.1 Hz, 14-H); δ_C see Table 1; *m/z* (CI) 439 ($[M + 1]^+$, 2%) and 379 ($[M + 1]^+ -$ AcOH, 62) (Found: C, 65.9; H, 8.7. $C_{24}H_{38}O_7$ requires C, 65.73; H, 8.73%); together with (13S,14S)-ent-6α,18-*diacetoxy*-14-*hydroxy*-8α,13; 15,16-*diepoxylabdane* (**8**) (60 mg, 6%) also as an oil; $[\alpha]_D$ −41° (*c* 1 in $CHCl_3$); ν_{max} 3 456 (OH), and 1 736 and 1 244 cm^{-1} (OCO); δ_H 0.83 and 0.87 (3 H, each, s, 4-Me and 10-Me), 1.40 (3 H, s, 8-Me), 1.99 and 2.06 (3 H each, s, AcO groups), 3.62 (2 H, s, 16-H), 3.57 and 4.02 (2 H, AB system, *J* 10.8 Hz, 18-2 H), 3.63 (1 H, dd, *J* 9.5 and 2.2 Hz) and 4.11 (1 H, dd, *J* 9.5 and 4.1 Hz) (15-2 H), 4.31 (1 H, dd, *J* 4.1 and 2.2 Hz, 14-H), and 5.02 (1 H, ddd, $J_1 = J_2$ 11.4 and J_3 3.7 Hz, 6-H); δ_C see Table 1; *m/z* (CI) 439 ($[M + 1]^+$, 1%) and 379 ($[M + 1]^+ -$ AcOH, 100) (Found: C, 65.6; H, 8.5. $C_{24}H_{38}O_7$ requires C, 65.73; H, 8.73%); and a mixture of products (40 mg) which, after acetylation with pyridine–Ac_2O (1:0.5 ml) at room temperature for 12 h, gave ent-3β,6α,16,18-*tetra-acetoxy*-13-epi-*manoyl oxide* (**9**) (25 mg, 2%) as an oil: $[\alpha]_D$ − 51° (*c* 0.5 in $CHCl_3$); ν_{max} 3 080 (C=C) and 1 736 and 1 238 cm^{-1} (OCO); δ_H 0.85 and 0.87 (3 H each, s, 4-Me and 10-Me), 1.32 (3 H, s, 8-Me), 1.97, 2.01, 2.05, and 2.10 (3 H each, s, AcO groups), 3.80 (2 H, collap. AB system, 16-2 H), 3.54 and 4.01 (2 H, AB system, *J* 11.1 Hz, 18-2 H), 4.82 (1 H, dd, *J* 11.6 and 4.5 Hz, 3-H), 5.50–5.18 (3 H, part AB of an ABX system, 15-2 H and signal of 6-H), and 5.91 (1 H, part X of an ABX system, $J_{AX} + J_{BX}$ 28.0 Hz, 14-H); δ_C see Table 1; *m/z* (CI) 523 ($[M + 1]^+$, 34%) and 463 ($[M + 1]^+ -$ AcOH, 100) (Found: C, 64.2; H, 8.0. $C_{28}H_{42}O_9$ requires: C, 64.35; H, 8.10%).

Biotransformation of Substrate (**5**).—Substrate (**5**) (900 mg) was dissolved in EtOH (20 ml) and the solution distributed among 20 Erlenmeyer flask cultures. The latter were incubated for 5 days, after which they were processed as indicated above for the biotransformation of substrate (**3**), to give a mixture (730 mg) which was then chromatographed on silica gel column to give starting material (**5**) (235 mg, 25%) and ent-6α,18-*diacetoxy*-11β,16-*dihydroxymanoyl oxide* (**16**) (215 mg, 23%) as an oil: $[\alpha]_D$ −75° (*c* 1 in $CHCl_3$); ν_{max} 3 472 (OH), 3 086 (C=C), and 1 736 and 1 241 cm^{-1} (OCO); δ_H 0.85 (3 H, s, 4-Me), 1.26 (3 H, s, 10-Me), 1.63 (3 H, s, 8-Me), 1.99 and 2.04 (3 H each, s, AcO groups), 3.32 and 3.42 (2 H, AB system, *J* 11.2 Hz, 16-2 H), 3.65 and 3.98 (2 H, AB system, *J* 10.8 Hz, 18-2 H), 4.41 (1 H, ddd, $J_1 = J_2$ 7.0 and J_3 4.7 Hz, 11-H), 5.11 (1 H, ddd, $J_1 = J_2$ 11.3 and J_3 4.1 Hz, 6-H), 5.09–5.26 (2 H, part AB of an ABX system, 15-2 H) and 5.76 (1 H, dd, part X of an ABX system, $J_{AX} + J_{BX}$ 28.2 Hz, 14-H); δ_C see Table 1; *m/z* (CI) 439 ($[M + 1]^+$, 1%) and 379 ($[M + 1]^+ -$ AcOH, 85) (Found: C, 65.6; H, 8.8. $C_{24}H_{38}O_7$ requires C, 65.73; H, 8.73%); and (13R,14R)-ent-6α,18-*diacetoxy*-14-*hydroxy*-8α,13;15,16-*diepoxylabdane* (**17**) (80 mg, 9%) also as an oil: $[\alpha]_D$ −58° (*c* 1 in $CHCl_3$); ν_{max} 3 448 (OH) and 1 737 and 1 241 cm^{-1} (OCO); δ_H 0.83 and 0.87 (3 H each, s, 4-Me and 10-Me), 1.24 (3 H, s, 8-Me), 1.99 and 2.07 (3 H each, s, AcO groups), 3.48 (1 H, dd, *J* 8.9 and 6.3 Hz) and 3.97 (1 H, dd, *J* 8.9 and 7.0 Hz) (15-2 H), 3.60 and 4.03 (2 H, AB system, *J* 10.8 Hz, 18-2 H), 3.64 and 3.80 (2 H, AB system, *J* 8.7 Hz, 16-2 H), 4.09 (1 H, dd, *J* 7.0 and 6.3 Hz, 14-H) and 5.03 (1 H, ddd, $J_1 = J_2$ 11.3 and J_3 3.9 Hz, 6-H); δ_C see Table 1; *m/z* (EI) 438 (M^+, 7%), 378 ($M^+ -$ AcOH, 7) (Found: C, 65.6; H, 8.4. $C_{24}H_{38}O_7$ requires C, 65.73; H, 8.73%).

Acetylation of Metabolite (**8**).—Metabolite (**8**) (20 mg) was acetylated with pyridine–Ac_2O (0.5:0.25 ml) at room temperature for 24 h. After column chromatography (13S,14S)-ent-6α,14,18-*triacetoxy*-8α,13;15,16-*diepoxylabdane* (**10**) (16 mg, 73%) was isolated.

Epoxidation of Compound (**3**).—Compound (**3**) (200 mg) was dissolved in $CHCl_3$ (15 ml) and epoxidized with MCPBA (250 mg) at room temperature for 48 h. Silica gel column chromatography of the mixture gave starting material (**3**) (50 mg, 25%), (13S,14S)-ent-6α,18-*diacetoxy*-16-*hydroxy*-8α,13; 14,15-*diepoxylabdane* (**11**) (25 mg, 12%) together with a mixture (100 mg, 48%) of the epoxide (**11**) and its epimer (13S,14R)-ent-6α,18-*diacetoxy*-16-*hydroxy*-8α, 13; 14,15-*diepoxylabdane* (**12**) which was impossible to separate.

Cyclization of the Epoxide (**11**).—The epoxide (**11**) (15 mg) was dissolved in $CHCl_3$ (5 ml) and Al_2O_3 (30 mg) was added. After the mixture had been heated under reflux for 6 h, it was filtered and subjected to column chromatography to give a compound (12 mg, 80%) identical with metabolite (**8**).

Cyclization of the Epoxides (**11**) *and* (**12**).—The mixture of epoxides (**11**) and (**12**) (100 mg) was dissolved in $CHCl_3$ (15 ml) and Al_2O_3 (100 mg) was added. After the mixture had been stirred for 6 h under reflux, it was filtered and chromatographed on a silica gel column to give a compound (35 mg, 35%) identical with metabolite (**8**) and (13S,14R)-ent-6α,18-*diacetoxy*-14-*hydroxy*-8α, 13; 15,16-*diepoxylabdane* (**13**) (45 mg, 45%) as an oil: $[\alpha]_D$ −65° (*c* 1 in $CHCl_3$); ν_{max} 3 483 (OH) and 1 736 and 1 241 cm^{-1} (OCO); δ_H 0.83 and 0.90 (3 H each, s, 4-Me and 10-Me), 1.41 (3 H, s, 8-Me), 1.99 and 2.07 (3 H each, s, AcO groups), 3.57 and 3.65 (2 H, AB system, *J* 8.3 Hz, 16-2 H), 3.58 and 4.04 (2 H, AB system, *J* 10.8 Hz, 18-2 H), 3.73–3.81 (2 H, part AB of an ABX system, 14-H and 15-H^1), 3.97 (1 H, dd, part X of an ABX system, $J_{AX} + J_{BX}$ 13.3 Hz, 15-H^2) and 5.02 (1 H, ddd, $J_1 = J_2$ 11.3 and J_3 3.9 Hz, 6-H); δ_C see Table 1; *m/z* (EI) 438 (M^+, 14%) and 378 ($M^+ -$ AcOH, 12) (Found: C, 65.6; H, 8.9. $C_{24}H_{38}O_7$ requires C, 65.73; H, 8.73%).

Acetylation of Compound (**13**).—Compound (**13**) (30 mg) was dissolved in pyridine–Ac_2O (0.5:0.25 ml) and the solution set aside at room temperature for 24 h. After this the mixture was chromatographed on silica gel column to give (13S,14R)-ent-6α,14,18-*triacetoxy*-8α,13; 15,16-*diepoxylabdane* (**14**) (23 mg, 70%).

Acetylation of Metabolite (**17**).—Metabolite (**17**) (30 mg) was acetylated with pyridine–Ac_2O (1:0.5 ml) at room temperature for 24 h. Column chromatography of the reaction mixture gave (13R,14R)-ent-6α,14,18-*triacetoxy*-8α,13; 15,16-*diepoxylabdane* (**18**) (20 mg, 61%).

Epoxidation of Compound (**5**).—Compound (**5**) (200 mg) was dissolved in $CHCl_3$ (20 ml) and epoxidized with MCPBA (200 mg) for 24 h at room temperature to give, after column chromatography, starting material (**5**) (40 mg, 20%), (13R,14S)-ent-6α,18-*diacetoxy*-16-*hydroxy*-8α,13; 14,15-*diepoxylabdane* (**19**) (75 mg, 36%) and (13R,14R)-ent-6α,18-*diacetoxy*-16-*hydroxy*-8α,13; 14,15-*diepoxylabdane* (**20**) (63 mg, 30%).

Cyclization of the Epoxide (**19**).—The expoxide (**19**) (60 mg) was dissolved in $CHCl_3$ (20 ml) and Al_2O_3 (70 mg) was added. After the mixture had been heated under reflux for 6 h it was filtered and subjected to column chromatography to give (13R,14S)-ent-6α,18-*diacetoxy*-14-*hydroxy*-8α,13;15,16-*diepoxy-labdane* (**21**) (45 mg, 75%) as an oil: $[\alpha]_D$ −38° (*c* 1 in $CHCl_3$); ν_{max} 1 736 and 1 243 cm^{-1} (OCO); δ_H 0.83 and 0.88 (3 H

each, s, 4-Me and 10-Me), 1.26 (3 H, s, 8-Me), 2.01 and 2.07 (3 H each, s, AcO groups), 3.58–3.76 [5 H, part B of an AB system, J 10.8 Hz (18-H[1]), part AB of an ABX system (14-H and 15-H[1]) and AB system, J 7.9 Hz (16-2 H)], 3.90 (1 H, dd, part X of an ABX system, $J_{AX} + J_{BX}$ 14.4 Hz, 15-H[2]), 4.04 (1 H, part A of an AB system, J 10.8 Hz, 18-H[1]), and 5.05 (1 H, ddd, $J_1 = J_2$ 11.3 and J_3 5.0 Hz, 6-H); δ_C see Table 1; m/z (EI) 438 (M^+, 4%) (Found: C, 65.6; H, 8.7. $C_{24}H_{38}O_7$ requires C, 65.73; H, 8.73%)

Cyclization of the Epoxide (**20**).—The epoxide (**20**) (50 mg) was dissolved in $CHCl_3$ (60 ml) and Al_2O_3 (60 mg) was added. After the mixture had been stirred for 6 h under reflux it was filtered and chromatographed on a silica gel column to give a compound (35 mg, 70%) identical with metabolite (**17**).

Acetylation of Compound (**21**).—Compound (**21**) (25 mg) was acetylated with pyridine–Ac_2O (1:0.5 ml) for 24 h at room temperature. After column chromatography (13R,14S)-ent-6α,14; 18-*triacetoxy*-8α,13; 15,16-*diepoxylabdane* (**22**) (17 mg, 62%) was isolated.

Epoxides (**11**), (**19**), *and* (**20**) *and the Triacetates* (**10**), (**14**), (**18**) *and* (**22**).—Spectral results (IR, ^1H and ^{13}C NMR, and mass), together with the optical rotations and elemental analyses for these compounds are available as a Supplementary publication [SUP No. 56780 (7 pages)]. For details of the Supplementary publications scheme, see 'Instructions for Authors (1990)', *J. Chem. Soc., Perkin Trans 1*, 1990, Issue 1.

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14 Colección Española de Cultivos Tipo (CECT), Departamento de Microbiología, Universidad de Valencia, Spain.

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