Biotransformation of highly substituted ent-kaur-16-enes by Rhizopus nigricans

ARTICLE *in* PHYTOCHEMISTRY · DECEMBER 1990

Impact Factor: 2.55 · DOI: 10.1016/0031-9422(90)89024-4

6 AUTHORS, INCLUDING:

Antonio Martínez
University of Granada
70 PUBLICATIONS **577** CITATIONS

SEE PROFILE

Phytochemistry, Vol 29, No 1, pp 121–126, 1990
Printed in Great Britain

0031-9422/90 $3.00+0.00

BIOTRANSFORMATION OF HIGHLY SUBSTITUTED *ENT*-KAUR-16-ENES BY *RHIZOPUS NIGRICANS*

A. GARCÍA-GRANADOS,* A. MARTÍNEZ, M. E. ONORATO,† M. L RUIZ, J M SÁNCHEZ and J M. ARIAS‡

Departamento de Química Orgánica, Facultad de Ciencias, †Análisis y Determinación de Estructuras, Servicios Técnicos, ‡Departamento de Microbiología, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain

(*Received in revised form* 26 *April* 1989)

Key Word Index—*Rhizopus nigricans*, fungus, tetrasubstituted *ent*-kaur-16-enes, biotransformation, metabolites, structural determination

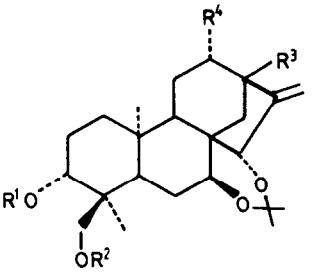
Abstract—The behaviour of *Rhizopus nigricans* in the biotransformation of 3,7,15,18-tetrasubstituted *ent*-kaur-16-enes has been studied. Incubation of *ent*-18-acetoxy-3β-hydroxy-7α,15β-isopropylidenedioxykaur-16-ene by *R nigricans* for 48 hr yielded mainly a 13-hydroxyl derivative. Incubation for eight days produced *ent*-16β,17-epoxidation, hydroxylation at C-12 and C-13, and *ent*-16α,17-dihydroxylation. Occasionally *R. nigricans* deacetylated and hydrolysed the isopropilidene group. The structure of the metabolites were determined by both spectroscopic (mainly by monodimensional and bidimensional NMR) and chemical means.

INTRODUCTION

There is very little data available on the biotransformation of *ent*-kaur-16-enes by hydroxylating fungi, apart from that on *Giberella fujikuroi* [1–7]. We are currently studying the behaviour of *Rhizopus* and *Aspergillus* strains [1, 2] and have observed that these fungi metabolize mainly the original C-16/C-17 double bond and the C-3 position of substrates substituted at C-3, C-7 and C-18 [1, 2] To determine the relationship between the structure of the substrate and the site where the fungal enzymes act we have now incubated a highly functionalized *ent*-kaur-16-ene with *R. nigricans*.

RESULTS AND DISCUSSION

The *ent*-18-acetoxy-3β,7α,15β-trihydroxykaur-16-ene (18-acetylleucanthol, (**1**) is a common diterpenoid within the genus *Sideritis* [8–11] Treatment of **1** with 2,2-dimethoxypropane gave its 7,15-isopropylidene derivative **2** [12], which was obtained to reduce its chromatographic polarity Incubation of **2** with *R nigricans* for 48 hr gave three products (**3**–**5**), mainly metabolite **3** (22%) When metabolite **3** was kept in solution in chloroform, product **4** was formed As acetylation of **3** and **4** gave the same diacetate (**6**), product **4** must be the result of the usual migration of an acetate group in this type of system [1] ^{1}H NMR spectra of **3** showed the same functional group at C-3, C-7, C-15, C-18 and the C-16 double bond as substrate **2** The proton signals at C-17, however, were different from those shown for the substrate (δ5.40, *d*, J=2 Hz and δ5.30, **s**, 1H each for **3** and δ5.15, *br s*, 2H for **2**) Moreover, the proton at C-13 in **2** (δ2.75, *m*, $W_{1/2}$=7 Hz, 1H) was not present in the ^{1}H NMR spectrum of **3** For comparative purposes substrate **2** was acetylated to give **7** ^{13}C NMR for products **6** and **7** confirmed that a new hydroxylation was introduced at C-13 on substrate **2** (see Table 1). A C-13 hydroxylation was indicated during the incubation of 7α- and 7β-hydroxykaurenolide [3]. Metabolite **5** was the result of deacetylation of substrate **2**, which was proved by comparing it with its 3,18-isopropylidene derivative (**8**), which was also obtained by acetonation of the simplest natural product leucanthol **9** [12, 13]. Thus, the

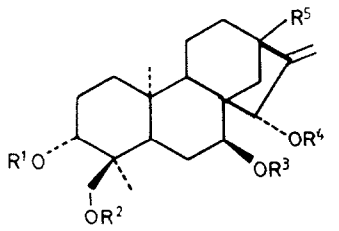


	R^1	R^2	R^3	R^4
2	H	Ac	H	H
3	H	Ac	OH	H
4	Ac	H	OH	H
5	H	H	H	H
6	Ac	Ac	OH	H
7	Ac	Ac	H	H
8	$-CMe_2-$		H	H
13	Ac	Ac	H	OAc
15	Ac	Ac	OAc	H

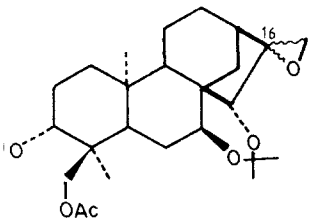
*Author to whom correspondence should be addressed

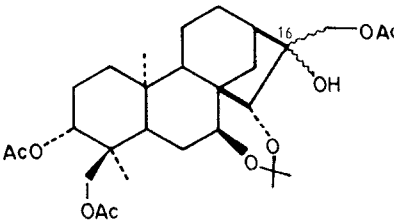
Table 1 ^{13}C NMR chemical shifts of compounds **2**, **6**, **7**, **13**, **15** and **16**

C	**2**	**6**	**7**	**13**	**15**	**16**
1	37 71	37 06	37 33	37 09	37 23	37 83
2	24 48	22 80	23 03	22 90	22 95	23 56
3	72 94	74 66	74 96	74 80	74 82	73 56
4	41 58	40 04	40 21	40 19	40 03	40 38
5	40 19	39 77	39 91	39 66	40 20	39 46
6	26 45	24 02	24 19	24 29	24 15	23 00
7	72 12	71 42	72 02	71 71	71 52	73 83
8	48 84	46 25	48 81	48 21	47 39	48 60
9	51 11	49 54	51 07	51 34	49 63	47 30
10	37 21	36 78	37 13	36 15	36 79	38 73
11	17 25	18 71	17 28	22 90	18 79	19 76
12	36 27	43 33	36 27	73 48	38 99	37 73
13	43 22	79 22	43 21	46 90	86 43	86 05
14	33 09	38 83	33 08	29 80	35 57	36 13
15	80 71	79 11	80 69	80 68	78 10	77 83
16	154 24	154 57	154 14	149 81	151 29	152 09
17	109 98	109 70	110 03	113 30	111 35	111 89
18	66 57	64 50	64 73	64 66	64 68	64 87
19	11 18	12 20	12 35	12 42	12 30	13 12
20	17 31	16 97	17 28	15 10	17 04	18 00
$C(Me)_2$	101 31	101 43	101 01	101 33	101 71	
C(Me)	24 83	24 67	24 89	24 66	24 71	
	23 69	23 47	23 67	23 66	23 60	
COMe	171 38	171 01	170 68	171 16	171 16	170 71
		170 59	170 25	170 73		170 69
						170 00
						169 80
COMe	21 05	21 18	21 33	21 62	22 16	22 12
		20 81	20 96	21 33	21 33	21 33
				20 98	20 95	21 22
						21 01



	R^1	R^2	R^3	R^4	R^5
1	H	Ac	H	H	H
9	H	H	H	H	H
12	Ac	Ac	Ac	Ac	H
16	Ac	Ac	Ac	Ac	OAc
17	Ac	Ac	Ac	Ac	OH

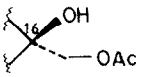




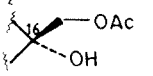
10

11

14



18



biotransformation of substrate **2** by *R. nigricans* resulted mainly in hydroxylation at C-13.

Incubation with *R. nigricans* under the same conditions as used for substrate **2** for eight days caused more extensive metabolism. The spectroscopic behaviour of metabolite **10**, isolated from this incubation (6%), suggested epoxidation of the original double bond of substrate **2**. Thus, its ^{1}H NMR spectrum showed, in addition to the expected signals for functional groups in 2, the loss of the unsaturated exocyclic methylene, but a new AB system at δ2.89 and 2.85 (2H, AB system, J=5.03 Hz). A new oxygen [mass spectrum *m/z* 435, $[M+1]^+$, chemical ionization] and two new oxygenated carbons [^{13}C NMR spectroscopy (δ65.48, C-16 and 48.68 C-17)] were also detected, all of which suggested the presence of a 16,17-epoxide group. The configuration at C-16 was not easy to determine. Chemical epoxidation of substrate **2** (see Experimental), yielded products **10** and **11**, which proved to be epimers at C-16. In this case product **11** showed the AB system of 2H-17 as two doublet signals at δ3.13 and 2.78 (2H, J=5.79 Hz). The chemical shifts of H-15 in **10** (δ3.56) were more deshielded than that in **11** (δ3.40), and the proton at C-17 in **11** (δ3.13) was more deshielded than the corresponding one in **10** (δ2.89), indicating an *ent*-16β configuration for **10** and *ent*-16α for **11**. Furthermore, NOE-difference experiments were performed for both **10** and the C-16 epimer **11**. Irradiation at H-15 of **10** produced an increase in the intensity of the most deshielded signal of the epoxide protons (7%) as well as a small but clear positive NOE for the other epoxide proton. This NOE was confirmed by the inverse experiment. Similar irradiation at H-15 of **11** did not produce a NOE-effect on any epoxide proton.

In addition to **10** a mixture of more polar metabolites was isolated from the *R. nigricans* culture. To separate them the mixture was acetylated, yielding **6** (3%), **12** (5%), **13** (2%), **14** (2%), **15** (1.6%), **16** (9%) and **17** (4%).

Product **12** was identified as tetraacetylleucanthol [13] and was the result of acetylation of the hydrolysed substrate **2**. Product **13** had a M_r of 518 (*m/z* 519, $[M+1]^+$, chemical ionization). This product showed ^{1}H NMR signals similar to those found in diacetate **7**, but in addition, a new proton at δ4.81, geminal to an acetoxyl group could be seen overlapping the proton at C-3. ^{13}C NMR spectra of **13** showed, in addition to the oxygenated C-3, C-7, C-15 and C-18, a new signal for an oxygenated carbon at δ73.48. These chemical shifts and consideration of probable effects on surrounding carbons lead us to propose the C-12 position for this new function. A C-12 hydroxylation was indicated on incubation of 7α-hydroxykaurenolide with *R. arrhizus* [3]. The configuration at C-12 was difficult to determine because ring C of **13** is not very rigid and two conformational situations may be present, depending on the configuration at C-12. A *J*-resolved 2D NMR experiment allowed us to separate the proton signals at C-3 and C-12. The coupling constants (J_1=5.75, J_2=3.89, J_3=1.90 Hz) shown by H-12, and a γ-effect produced on C-14, prompt us to propose an *ent*-12β configuration for this acetoxy group, ring C being in a twist-boat conformation. The coupling constants agree relatively well with those described for this configuration at C-12 of *ent*-kaur-16-enes [14, 15].

One further product isolated from the metabolite acetylation mixture was **14**, which had a M_r of 536 (*m/z* 537, $[M+1]^+$, chemical ionization). Its ^{1}H NMR spectrum showed three acetoxyl groups, one isopropilidenedioxy group and C-19 (δ0.77) and C-20 (δ0.99) methyl singlet groups. The presence of two acetoxymethylene groups (AB systems with doublet centred at δ4.30 and 4.17 (J=12.14 Hz) and 3.98 and 3.47 (J=11.76 Hz) and the absence of an olefin proton signify that an acetoxyl group must be situated at C-17, with possibly a hydroxyl group at C-16. ^{13}C NMR spectra of **14** confirmed this hypothesis, showing two primary and another tertiary oxygenated carbon (δ67.23 and 81.41, respectively). Thus, *R. nigricans* produced hydroxylation at C-16 and C-17 of substrate **2**, as observed on previous occasions [2, 3]. To determine the configuration at C-16 we hydroxylated **7** with osmium tetroxide to give products **14** (34%) and **18** (25%). Probably due to the function at C-15 of **7** the reaction is not so specific as found in other *ent*-kaur-16-enes [16, 17]. We ascertained the configuration of C-16 for both compounds (**14** and **18**) with the aid of NOE-difference experiments. Irradiation of 18 at H-15 produced considerable NOE-effects on the 2H-17 AB system, which were not seen with a similar irradiation of **14** at H-15. The chemical shifts of H-15 in **18** was lower (δ3.36) than that in **14** (δ3.56). This difference in chemical shift would appear to indicate these configurations, but we think it is more accurate to determine these configurations by NOE-difference experiments than by ^{1}H NMR chemical shifts alone. ^{13}C chemical shifts of **14** and **18** were not conclusive for the determination of these particular configurations except for the chemical-shifts values for H-15 in both epimers at C-16. Thus, the γ-effect on C-15 of the acetoxy group at C-17 of **18** was well transmitted through the proton at C-15. This γ-effect was not efficiently produced in product **14**, due to the proximity of both electronegative groups at C-15 and C-18 [18]. *Rhizopus nigricans* therefore produced epimer configurations at C-16 for epoxide (**10**) and for glycol (**14**).

Products **15–17** were also isolated from the mixture resulting from acetylation of polar metabolites. Product **15** was identified as peracetylated **3**. In this case, the fungus introduced a hydroxyl group at C-13, as it did in the 48 hr incubation. Acetylation of **17** gave **16**. Compounds **15–17** are essentially the result of C-13 hydroxylation but in the case of **16** and **17** hydrolysis of the isopropylidenedioxy groups also occurred in the medium.

The results of our incubations indicate that the fungal enzyme activity is directed towards positions 12, 13, 16 and the 17 of the molecule **2**, as observed for *R. arrhizus* in the case of 7α and 7β-kaurenolide [3].

EXPERIMENTAL

^{1}H NMR spectra were measured in $CDCl_3$ solns at 80 and 300 MHz. ^{13}C NMR spectra were determined at 75.47 MHz in $CDCl_3$ soln (which also provided the lock signal). Assignments of ^{13}C chemical shifts were made with the aid of distortionless enhancement by polarization transfer (DEPT) using a flip angle of 135°. Monodimensional NOE-difference experiments were performed by irradiation for 4 sec in a series of 8 scans, alternatively on and off resonance. 2D-C/H correlation expts were carried out as described in refs [19–21], with 2.941 Hz/point in F2 and 13.197 Hz/point in F1 and 2D-JRES as described in ref. [22] with 0.122 Hz/point in F2 and 0.488 Hz/point in F1. MS were recorded using CI (methane). Silica gel Sharlau 60 (less than 0.06 mm) was used for flash chromatography. CH_2Cl_2 with increasing amounts of Me_2CO was used as eluent. TLC plates (silica gel Merck G) were

visualized by spraying with H_2SO_4–HOAc–H_2O, followed by heating at 120°

Isolation of **1**. The *ent*-18-acetoxy-3β,7α,15β-trihydroxykaur-16-ene (18-acetylleucanthol) **1** [8, 11] was isolated from *S granatensis* var *nijarensis* [9]

Treatment of **1** with *2,2-dimethoxypropane* Product **1** (1 5 g) was dissolved in 2,2-dimethoxypropane (25 ml) and refluxed for 2 hr with pyridine *p*-toluensulphonate (50 mg) The mixt was concd under vacuum, washed with H_2O, extd with CH_2Cl_2 and dried ($MgSO_4$), yielding after CC, 1 2 g of *ent*-18-acetoxy-3β-hydroxy-7α,15β-isopropylidenedioxykaur-16-ene (**2**) [12] ^{1}H NMR (δ80 MHz) 5 15 (2H, *br* s, 2H-17), 4 08 and 3 88 (2H, AB system, J = 12 Hz, 2H-18), 3 84 (1H, *br* s, H-15), 3 50 (2H, *m*, $W_{1/2}$ = 16 Hz, H-3 and H-7), 2 75 (1H, *m*, $W_{1/2}$ = 7 Hz, H-13), 2 05 (3H, s, AcO), 1 38 and 1 32 (3H each, s, Me_2C), 1 0 (3H, s, 3H-20) and 0 75 (3H, *s*, 3H-19) ^{13}C NMR see Table 1

Organism, media and culture conditions *Rhizopus nigricans* CECT 2672 (ATCC 10404) was obtained from Colección Española de Cultivos Tipo [23] Medium YEPGA containing 1% yeast ext, 1% peptone, 2% glucose, 2% agar, at pH 5 was used for storage of *R nigricans* In the transformation expts a medium composed of 0 1% peptone, 0 1% yeast ext, 0 1% beef ext and 0 5% glucose at pH 5 7 in H_2O was used Erlenmeyer flasks (250 ml) containing 60 ml of medium were inoculated with a dense suspension of *R nigricans* Incubations were maintained at 28° with gyratory shaking (150 rpm) for 6 days Substrate **2** (1 g) was dissolved in EtOH (20 ml), distributed among 20 Erlenmeyer flask cultures and incubated for 48 hr, after which eight flasks were sepd from incubation Incubation of the other 12 flasks was maintained for 8 days

Recovery and purification *48 hr Incubation* Cultures were filtered and pooled, and cells washed × 2 with H_2O The liquid was satd with NaCl and extracted with CH_2Cl_2 These extracts were dried ($MgSO_4$) and evapd at 40° *in vacuo*, giving 260 mg of mixt products After CC, 40 mg of substrate **2**, 90 mg of *ent*-18-acetoxy-3β,13-dihydroxy-7α,15β-isopropylidenedioxykaur-16-ene (**3**), 30 mg of *ent*-3β-acetoxy-13,18-dihydroxy-7α,15β-isopropylidenedioxykaur-16-ene (**4**) were isolated In addition, 50 mg of a polar mixt isolated from this incubation was treated with 2,2-dimethoxypropane (5 ml) and refluxed for 2 hr with pyridine *p*-toluensulphonate (5 mg) After CC, 30 mg of *ent*-3β,18-7α,15β- diisopropylidenedioxykaur-16-ene (**8**) [12, 13] was isolated 8 *Day Incubation* Proceeding as described for the 48 hr incubation, 15 mg of substrate **2**, 26 mg of *ent*-18-acetoxy-3β-hydroxy-7α,15β- isopropylidenedioxy-16β,17-epoxykaurane (**10**) and 320 mg of a mixt of polar products was isolated This mixt was acetylated with pyridine–Ac_2O (2 1), refluxed for 5 hr, poured into cold H_2O (100 ml) and then extracted with CH_2Cl_2 (3 × 50 ml) The organic layer was washed with aq $KHSO_4$ (2 × 25 ml), H_2O, dried ($MgSO_4$) and concd *in vacuo* After CC, 20 mg of product **7**, 35 mg of *ent*-3β,7α,15α,18-tetraacetoxykaur-16-ene (**12**) [13], 16 mg of *ent*-3β,12,18-triacetoxy-7α,15β-isopropylidenedioxykaur-16-ene (**13**), 18 mg of *ent*-16α-hydroxy-3β,17,18-triacetoxy-7α,15β-isopropylidenedioxykaurane (**14**), 12 mg of *ent*-3β,13,18-triacetoxy-7α,15β-isopropylidenedioxykaur-16-ene (**15**), 76 mg of *ent*-3β,7α,13,15β,18-pentaacetoxykaur-16-ene (**16**) and 32 mg of *ent*-13-hydroxy-3β,7α,15β,18-tetraacetoxykaur-16-ene (**17**) were isolated

Product **3** Gum, $[\alpha]_D$ −46° ($CHCl_3$, *c* 1), ^{1}H NMR (δ80 MHz) 5 40 (1H, *d*, J = 2 Hz) and 5 30 (1H, s), (2H-17), 4 08 and 3 90 (2H, AB system, J = 12 Hz, 2H-18), 3 85 (1H, *m*, *br* s, H-15), 3 58 (1H *m*, $W_{1/2}$ = 6 Hz, H-7), 2 05 (3H, s AcO), 1 40 and 1 38 (3H each, s, Me_2C), 1 00 (3H, s, 3H-20) and 0 75 (3H, s, 3H-19)

Product **4** Gum, ^{1}H NMR (δ, 80 MHz) 5 35 (1H, *d*, J = 2 Hz) and 5 28 (1H, *br* s) (2H-17), 4 98 (1H, *dd*, J_1 = 11, J_2 = 5 Hz, H-3), 3 91 (1H, *br* s, H-15), 3 60 (1H, *m*, $W_{1/2}$ = 6 Hz, H-7), 3 38 and 2 98 (2H, AB system, J = 12 Hz, 2H-18), 2 08 (3H, s, AcO), 1 39 and 1 34 (3H each, *s*, Me_2C), 1 02 (3H, s, 3H-20) and 0 70 (3H, *s*, 3H-19)

Product **10** Gum, $[\alpha]_D$ = −36° ($CHCl_3$, *c* 1), IR ν_{max} (neat, cm^{-1}) 3275, 1742, 1380, 1226, 1043 and 920, ^{1}H NMR (δ300 MHz) 4 02 and 3 91 (2H, AB system, J = 11 5, 2H-18), 3 60 (1H, *dd*, $J_1 = J_2$ = 3 02 Hz, H-7), 3 56 (1H, s, H-15), 3 53 (1H, *dd*, J_1 = 10 68, J_2 = 5 80, H-3), 2 89 and 2 85 (2H, AB system, J = 5 03 Hz, 2H-17), 2 04 (3H, s, AcO), 1 36 and 1 27 (3H each, *s*, Me_2C), 0 99 (3H, s, 3H-20) and 0 73 (3H, s, 3H-19) ^{13}C NMR see Table 2 MS *m/z* (%) 435 $[M+1]^+$ (22), 417 (29), 377 (29), 359 (64), 341 (24), 317 (26), 299 (100), 281 (23)

Product **13** Gum, $[\alpha]_D$ = −32° ($CHCl_3$, *c* 0 5), IR ν_{max} (neat, cm^{-1}) 1739, 1375, 1241, 1036 and 905, ^{1}H NMR (δ, 300 MHz) 5 35 and 5 26 (1H each, *s*, 2H-17), 4 81 (1H, *ddd*, J_1 = 5 75, J_2 = 3 89, J_3 = 1 90 Hz, H-12), 4 80 (1H, *dd*, J_1 = 11 7, J_2 = 4 42 Hz, H-3), 3 98 and 3 51 (2H, AB system, J = 11 Hz, 2H-18), 3 87 (1H, *s*, H-15), 3 55 (1H, *m*, $W_{1/2}$ = 7 Hz, H-7), 2 86 (1H, *m*, $W_{1/2}$ = 10 Hz, H-13), 2 04, 2 0 and 1 99 (3H each, s, AcO), 1 35 and 1 31 (3H each, *s*, Me_2C), 1 09 (3H s, 3H-20) and 0 82 (3H, s, 3H-19) ^{13}C NMR see Table 1 MS *m/z* (%) 519 $[M+1]^+$ (2), 461 (20), 401 (34), 383 (12), 341 (20), 281 (10)

Product **14** Gum, $[\alpha]_D$ = −20° ($CHCl_3$, *c* 0 5), IR ν_{max} (neat, cm^{-1}) 3447, 1740, 1376, 1243 and 1039, ^{1}H NMR (δ, 300 MHz) 4 81 (1H, *dd*, J_1 = 11 64, J_2 = 4 68 Hz, H-3), 4 30 and 4 17 (2H, AB system, J = 12 14 Hz, 2H-17), 3 98 and 3 47 (2H, AB system, J = 11 76, 2H-18), 3 56 (1H, *d*, J = 2 Hz, H-15), 3 40 (1H, *dd*, $J_1 = J_2$ = 2 93 Hz, H-7), 2 08, 2 02 and 2 01 (3H each, s, AcO), 1 26 and

Table 2 ^{13}C NMR chemical shifts of compounds **10**, **11**, **14** and **18**

C	**10**	**11**	**14**	**18**
1	37 73	37 67	37 34	37 27
2	24 43	24 24	22 99	22 95
3	72 84	72 84	74 90	74 79
4	41 58	41 58	40 13	40 15
5	40 11	39 23	39 44	39 40
6	26 44	26 44	23 92	24 06
7	71 87	72 03	71 65	71 97
8	49 97	48 37	48 32	48 74
9	50 75	50 96	51 13	50 86
10	37 28	37 29	37 27	37 27
11	18 11	17 69	17 27	17 29
12	35 17	34 72	27 12	26 65
13	42 45	40 06	40 36	44 19
14	29 43	27 82	34 63	34 38
15	79 84	82 36	88 07	81 22
16	65 48	67 29	81 41	78 86
17	48 68	51 85	67 23	68 00
18	66 46	66 39	64 63	64 63
19	11 21	11 21	12 41	12 42
20	17 50	17 40	17 17	17 29
$C(Me)_2$	101 59	101 29	100 88	101 29
$(Me)_2C$	24 66	24 69	24 98	24 97
	23 27	23 62	23 47	23 82
COMe	171 36	171 43	171 35	171 42
			170 74	171 09
				170 72
COMe	21 01	21 02	21 31	21 31
			21 12	20 95
			20 94	

1 23 (3H each, s, Me2C), 0 99 (3H, s, 3H-20) and 0 77 (3H, s, 3H-19). ISCNMR. see Table 2 MS *m/z* (%) 537 [M+ 1] + (10), 477 (7), 461 (8), 419 (9), 401 (6), 359 (6), 341 (6), 281 (4), 60 (100)

Product 15. Gum, IR Vma~(neat, cm- 1) 3082, 1738, 1372~1244, 1035 and 903, 1H NMR (6, 300 MHz) 5 38 (1H, d, J = 1.84) and 5,30 (1H, *br s)* (2H-17), 4 82 (1H, *dd*, J1 = 11 69, J2 =4.44 Hz, H-3), 3 97 and 3 52 (2H, AB system, J = 11 69 Hz, 2H-18), 3 78 (1H, d, J= 1.37 Hz, H-15), 3 53 (1H, m, WI/2 =6 Hz, H-7), 2 02 (6H, s) and 2 01 (3H, s) (AcO), 1 36 and 1 31 (3H each, s, MezC), 1 04 (3H, s, 3H-20) and 0 79 (3H, s, 3H-19) 13C NMR see Table 1 MS *m/z* (%)" 519 [M + 1] + (5), 461 (61), 401 (33), 383 (16), 341 (20), 281 (7), 60 (lOO)

Product 16 Gum, [~]D= +74° (CHCIs, c 1), IR ~max(neat, cm-1) 1738, 1372, 1039 and 916, IHNMR(6,300MHz) 535 and 524 (1H each, s, 2H-17), 5 32 (1H, br s, H-15), 500 (IH, m, *W1/2*= 7 Hz, H-7), 4 69 (IH. *dd*, J1 = 11 69, J2 =4 52 Hz, H-3), 3 97 and 3 46 (2H, AB system, J = 11 67 Hz, 2H-18), 2 64 (1H, *dd*, *J1 = 9 5, Jz* = 1 5 Hz, H- 13), 2 04 (3H), 2 0 (6H), 1 97 (3H) and 1 94 (3H) (s, AcO), 1 15 (3H, s, 3H-20) and 080 (3H, s, 3H-19) 13C NMR see Table l MS *m/z* (%) 563 [M + 1] + (1), 503 (100), 443 *(12)*, 383 (26), 323 (10)

Product 17 Gum, [~]D = +35 ° (CHCI3, c 1), IR Vm~ (neat, cm-1) 3480, 1737, 1372, 1251, 1038 and 912, XHNMR (6, 300 MHz) 5 35 and 5 25 (IH each, s, 2H-17), 5 30 (1H, s, H-I 5), 499 (IH, m, Wl/z=7Hz , H-7), 470 (IH, *dd*, J1=1l 66, J2 =4 45 Hz, H-3), 3 97 and 3 46 (2H, AB system, J = 11 63 Hz, 2H-18), 202 (6H), 1 97 (3H), 1 95 (3H) (s, AcO), 1 11 (3H, s, 3H-20) and082(3H, s, 3H-19) *MSm/z(%)* 521 [M+I] + (0.5),461 (8), 443 (0 5), 419 (05), 401 (2), 383 (1), 60 (100)

Acetylatton of 2 Substrate 2 (100 mg) was acetylated with pyndlne-AczO (2 1) for 12 hr at room temp After CC, 85 mg of *ent-3~,18-dIacetoxy-7g,15,8-1sopropyhdene&oxykaur-16-ene* (7) was isolated Gum, [~]D=+12 (CHCI3, cl), IR Vm~ (neat, cm-1). 1740, 1374, 1242, 1037 and 902, 1H NMR (6300 MHz) 5.18 and 5 *14(2H, s, 2H-17), 483(lH, dd, Jt= ll, J2= 5* Hz, H-3), 3 99 and 3 50 (2H, AB system, J = 11 5 Hz, 2H- 18), 3 85 ~1H, s, H-15),3 50 (IH, m, *WI/2=6Hz, H-7),275(1H, m, W1/z=IO* Hz, H-13), 201 and 2,0 (3H each, s, AcO), I 36 and 1 32 (3H each, s, Me2C), 1 0 (3H, s, 3H-20) and 0 80 (3H, s. 3H-19) 13C NMR see Table 1. MS *m/z* (%) 461 [M + 1] ~ (4), 403 (72), 385 (23), 443 (100), 325 (40), 283 (41), 265 (11), 251 (3)

Acetylatton of 3. Metabohte 3 (50mg) was acetylated as described for 2 After CC 35 nag of *ent-3/3,18-dmcetoxy-13-*hydroxy-7c~,15~-lsopropyhdene&oxykaur-16-ene (6) was isolated Gum, [~]D = --51 o (CHCI3' c 2), IR Vma~(neat, cm- 1) 3496, 1736, 1377, 1246, 1033 and 904, 1H NMR (6300 MHz) 5 28 and *5 19(1Heach, brs, 2H-17),478(1H, dd, Ja*= 11 5, J2=4 5 Hz, H-3), 3 93 and 3 45 (2H, AB system, J= 11 68 Hz, 2H-18), 3.79 (1H, *brs*, H-15), 347 (1H, m, W1/2=6 Hz, H-7), 198 (3H) and 197 (6H) (s, AcO), 1 31 and 1 26 (3H each, s, MezC), 094 (3H, s, 3H-20) and 0 74 (3H, s, 3H-19) 13C NMR see Table 1 MS *m/z (%)* 477 [M+ 1] + (6), 419 (39), 401 (45), 359 (100), 341 (48), 299 (47), 281 (19), 265 (4)

Aeetylatlon of 4 Metabohte 4 (30 rag) was acetylated as described for 2 After CC 22 mg of 6 was ~solated

Epox~datton of 2 Substrate 2 (50 rag) was dissolved in CHCI3 (5 ml) and epoxl&zed with MCPBA (100 mg) for 48 hr at room temp After CC, 16rag of 10 and 18mg of *ent-18-acetoxy-*3/~-hydroxy-7~,15/~-lsopropyhdenedloxy-16~, 17-epoxykaurane (11) were isolated *product* 11 Gum, [aiD= -37 ° (CHCI3, c0.5); IR vm,~ (neat, cm-1)- 3290, 1741, 1380, 1227, 1043 and 901; 1HNMR (6300 MHz) 401 and 3 92 (2H, AB system, J=1l 51, 2H- 18), 3 54 (1H, *dd*, Jl = 10 77, J2 = 5 65 Hz, H-3), 3 50 (1H, *dd*, J~ =Jz =2 Hz, H-7), 3 40 (IH, d, J =2 Hz, H-15), 3 13 and 2 78 (2H, AB system, J=5 79 Hz, 2H-17), 2.04 (3H, s, AcO), 1 28 and 1.23 (3H each, s, Me,C), 0 99 (3H, s, 3H-20) and 0 73 (3H, s, 3H-19). 13CNMR. see Table 2 MS *m/z* (%). 435 [M + 1] + (11), 417 (8), 377 (22), 359 (56), 341 (14), 317 (30), 299 (100), 281 (32)

Osmylatzon of 7. Product 7 (75 rag) was dissolved in MezCO (2 ml) and dry Et20 (1 ml), after which H~O2 (30%, 0 5 ml) and t-BuOH containing 0 5% w/w of OsO4 (0.25 ml) [24] were added The mlxt was stirred for 42 hr at room temp After concn *m vacuo*, the mlxt. was extracted repeatedly with CH2C12, dried (MgSO4) and coned *m vacuo* After CC 30 mg of 14 and 22 mg of *ent-3~,17,18-trmcetoxy-16~-hydroxy-7c~,15~-lsopropyhdene-*dloxykaurane (18) were obtained *Product* 18 Gum, [C~]o= - 41° (CHCI3, el), IR Vma,(neat, cm-1) 3508, 1739, 1376, t244 and 1043 1HNMR (6300MHz) 483 (1H, *dd*, J1=1175, J2 = 4 45 Hz, H-3), 4 37 and 4 02 (2H, AB system, d = 11 33 Hz, 2H-17), 3 98 and 3 47 (2H, AB system, J = 11 56 Hz, 2H-18), 3 48 (1H, *dd*, dl = J2 = 3 39 Hz, H-7), 3 36 (1H, br s, H- 15), 2.07, 2 01 and 2.0 (3H each, s, AcO), 1 32 and 1 28 (3H each, s, Me2C), 0 97 (3H, s, 3H-20) and 077 (3H, *s*, H-19) 13CNMR see Table 2. MS *m/z* (%) 537 [M+ 1] + (53), 519 (12), 477 (27), 461 (26), 419 (26), 401 (9), 359 (10), 341 (6), 281 (6), 60 (100)

Sapomficatlon ofl Product 1 (100 mg) was dissolved in 30 rnl of MeOH-H20-KOH (21 9.1 5) The mixt was stirred for 2 hr at room temp after which it was dll with H20 (20 ml), neutrallzed with 2 M HCI, extracted with CH2C12, dried (MgSO4) and concd under vacuum After CC 85 mg of *ent-3~,18-dIhydroxy-*7~,15/~-lsopropyhdenedioxykaur-16-ene (leucanthol, 9) [13] was isolated

lsopropyhdenedloxyderwatwe of 9 Product 9 (60 mg) was dissolved m 2,2-dImethoxypropane (10 ml) and refluxed for 2 hr with pyrldlne p-toluensulphonate (5 mg) After CC 50 mg of 8 was Isolated

Acetylauon of 17 Metabohte 17 (20 mg) was acetylated with pyndlne-Ac20 (2" 1) and refluxed for 10 hr. After CC 16 mg of 16 were isolated

Acknowledgements-- This work was supported by a grant from Comlsl6n Asesora de lnvestlgac16n C~entifica y T6cnlca We thank Isabel J Macdonald for her assistance in the translation of the text IR, MS and NMR and the final revision of the text were carried out by the "Servlcio de Anfilisis y Determinac16n de Estructuras" and "Servlcio de Traducci6n T6cmca", *respectJvely*, (Servlcios T6cnIcos de la Universidad de Granada, Spare)

REFERENCES

1 Gareia-Granados, A, Onorato, M E., S~tenz de Buruaga, A and Arias, J M. (1982) *An, Qmm* 78C, 287
2 Garcia-Granados, A, Martinez, A, Onorato, M E and Arias, J M. (1986)J *Nat Prod* 49, 126
3 Hanson, J R, Savona, G and Swerns, M, (1974) *J Chem Soc. Perkm I* 2001
4 Ellanes, G and Hanson, J R (1976)J *Chem Soc Perkm I* 1666
5 Bellby, J P., Ghlsalbertl, E. L, Jefferies, P R, Sefton, M A and Sheppard, P N (1973) *Tetrahedron Letters* 2589
6 Ghlsalberti, E L, Jefferles, P R, Sefton, M. A and Sheppard, P. N, (1977) *Tetrahedron* 33, 2451
7 Ghisalbertl, E L., Jefferies, P. R and Sefton, M A (1982) J *Chem. Res* (S) 8, (M) 166
8 Carraseal, M. I, Rabanal, R. M, Mhrquez, C and Valverde, S (1978) *An Qu~m,*74, 1547
9 Garcia-Granados, A, Llnaza, F, Martinez, A., Onorato, M E, Sfienz de Buruaga, A., Sfienz de Buruaga, J M. and Socorro, O (1983) *An. Qutm.* 79C, 460

10 Cabrera, E., García-Granados, A., Sâenz de Buruaga, A. and Sâenz de Buruaga, J. M. (1983) *Phytochemistry* **22**, 2779
11 López Gómez, M. A., Márquez, C., Rabanal, R. M. and Valverde, S. (1979) *An. Quim.* **75**, 911
12 García-Granados, A., Martínez, P. A. and Parra, A. (1982) *An. Quim.* **78C**, 410
13 García de Quesada, T., Rodríguez, B., Valverde, S. and Huneck, S. (1973) *An. Quim.* **69**, 757
14 Bohlmann, F., Jakupovic, J., King, R. M. and Robinson, H. (1980) *Phytochemistry* **19**, 863
15 Herz, W., Kulanthaivel, P. and Watanabe, K. (1983) *Phytochemistry* **22**, 2021
16 Hanson, J. R. (1966) *Tetrahedron* **22**, 1453
17 Morofushi, M., Yokota, T. and Takahashi, N. (1973) *Tetrahedron Letters* 789
18 Garcia-Granados, A., Martínez, A., Onorato, M. E. and Santoro, J. (1986) *Magn. Resn. Chem.* **24**, 853
19 Bax, (1983) *J. Magn. Reson.* **53**, 517
20 Rutar, V. (1984) *J. Magn. Reson.* **58**, 306
21 Wilde, J. A. and Bolton, P. H. (1984) *J. Magn. Reson.* **59**, 343
22 Aue, W. P., Karhan, J. and Ernst, R. R. (1976) *J. Chem. Phys.* **64**, 4226
23 Coleccion Española de Cultivos Tipo (CECT), Departamento de Microbiología, Facultad de Ciencias, Universidad de Valencia Spain
24 Daniels, R. and Fischer, I. (1963) *J. Org. Chem.* **28**, 320