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MICROBIAL TRANSFORMATION OF TETRACYCLIC DITERPENES: CONVERSION OF *ENT*-KAURENONES BY *CURVULARIA* AND *RHIZOPUS* STRAINS

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ABSTRACT.—We have biotransformed several *ent*-kaur-15-enones and *ent*-kaur-16-enones with *Curvularia lunata* and *Rhizopus nigricans* cultures. Incubation of *ent*-18-acetoxykaur-15-ene-3,7-dione with *C. lunata* led to its 17-hydroxy derivative, and with *R. nigricans* to 17-hydroxy and 13-hydroxy derivatives. Incubation of *ent*-18-acetoxykaur-15-en-3-one with *C. lunata* also gave the 17-hydroxyderivative, while incubation with *R. nigricans* produced 13-hydroxy- and 3,13-dihydroxyderivatives. Moreover, biotransformation of *ent*-18-acetoxykaur-16-ene-3,7-dione with *C. lunata* gave its (16*R*),17-epoxy, (16*R*),17- and (16*S*),17-dihydroxy, and (7*S*)-hydroxy derivatives. The structures of the metabolites have been determined by spectroscopy and chemical correlations.

As a continuation of our systematic studies on microbial transformations of terpenes (1–8), we now report the bioconversion of *ent*-kaur-15-en-7-one and -3,7-dione by *Curvularia lunata* and *Rhizopus nigricans* to determine the possible influence of the ketone group at C-3. Furthermore, we have biotransformed one *ent*-kaur-16-ene-3,7-dione with *C. lunata* cultures. These data together with previous results (1,2,4) help to discover the relationship between the structure of *ent*-kaurane substrates and the site at which the microorganism acts.

MATERIAL AND METHODS

PHYSICAL ANALYSES.—Mp's were determined on a Kofler apparatus and are uncorrected. ^{1}H-nmr spectra were measured at 300 MHz ($CDCl_3$, δ $CHCl_3$ = 7.25) in a Bruker AM-300. ^{13}C-nmr spectra were determined at 74.75 MHz also in $CDCl_3$ (δ $CHCl_3$ = 77.100). Assignments were made with the aid of distortionless enhancement by polarization transfer (DEPT), using a "flip angle" of 135°. Ir spectra were recorded on an Ft-ir Nicolet 20SX spectrometer. Mass spectra were measured at 70 eV in a Hewlett-Packard 5988 A spectrometer. Elemental analyses were measured in a Perkin-Elmer 240C analyzer. The rotatory powers were measured on a Perkin-Elmer 240 polarimeter at 20°.

ISOLATION OF STARTING MATERIAL.—The *ent*-18-acetoxy-kaur-16-ene-3β,7α-diol (linearol [**1**]) and *ent*-18-acetoxykaur-15-ene-3β,7α-diol (isolinearol [**2**]) were isolated from *Sideritis funkiana* Willk. (Labiatae) (9), and the *ent*-7α-acetoxykaur-15-en-18-ol (siderol [**3**]) was isolated from *Sideritis pusilla* (Lange) Pau ssp. *flavovirens* (Rouy) Malagarriga (Labiatae) (10), and *Sideritis arborescens* Salzw. ex Bentham ssp. *paulii* (Pau) P.W. Ball ex Heywood (Labiatae) (11).

SYNTHESIS OF SUBSTRATES.—Linearol [**1**] (2 g) was dissolved in Me_2CO (60 ml) and oxidized with Jones reagent (12). After cc, 1.8 g of *ent*-18-acetoxykaur-16-ene-3,7-dione [**4**] was obtained (4). Isolinearol [**2**] (0.6 g) was dissolved in Me_2CO (25 ml) and oxidized with Jones reagent. After cc, 480 mg of *ent*-18-acetoxykaur-15-ene-3,7-dione [**5**] was isolated as a gum: $[\alpha]_D$ −27° (*c* = 1, $CHCl_3$); ir ν max (neat) cm^{-1} 1744, 1697, 1376, 1219, 1039, 998; ^{1}H nmr (δ) 5.49 (1H, s, H-15), 4.15 and 3.75 (2H, AB system, *J* = 11.3 Hz, H_2-18), 1.97 (3H, s, AcO), 1.72 (3H, d, *J* = 1.6 Hz, Me-17), 1.32 and 1.00 (3H, s, Me-19 and Me-20); ^{13}C nmr see Table 1; ms *m/z* (%) $[M]^+$ 358 (78), 298 (18), 285 (19), 273 (11), 255 (20), 227 (7), 201 (7), 199 (10), 316 (17). Found C 73.4, H 8.6; $C_{22}H_{30}O_4$ requires C 73.71, H. 8.43%.

Siderol [**3**] (1 g) was saponified with MeOH-H_2O (70:30) (30 ml) and KOH (1.5 g) for 18 h at room

TABLE 1. ^{13}C-nmr Chemical Shifts of Compounds **4–8**, **10**, **11**, and **15–22**.

Carbon	Compound														
	4[a]	**5**	**6**	**7**	**8**	**10**	**11**	**15**	**16**	**17**	**18**[b]	**19**	**20**	**21**	**22**
C-1	37.37	37.39	39.89	39.72	39.49	37.37	38.38	37.40	37.38	37.68	37.45	39.74	39.65	39.67	37.49
C-2	34.68	34.69	25.00	24.91	24.19	35.01	35.34	34.68	34.65	35.05	35.01	24.37	24.93	20.62	22.88
C-3	212.41	212.74[c]	35.10	35.81	35.66	216.17	214.55	212.25[c]	212.09[c]	216.63	216.46	35.19	35.10	35.67	73.61
C-4	50.00	50.10	39.31	39.38	38.63	52.21	49.73	50.15	50.16	52.43	52.44	38.75	38.72	38.70	40.84
C-5	45.95	45.99	44.05	43.94	49.12	45.23	39.80	46.05	46.16	45.76	45.48	49.42	49.02	48.06	45.56
C-6	37.01	36.89	26.27	26.95	37.10	37.05	25.95	36.82	37.00	37.26	37.21	37.06	36.99	37.13	36.35
C-7	211.37	212.40[c]	75.46	74.89	213.99	210.82	78.55	211.95[c]	210.75[c]	213.22	213.38	215.22	215.27	212.18	211.53
C-8	57.07	61.85	53.52	53.23	62.12	57.83	46.69	61.60	59.66	61.73	61.33	62.45	61.99	60.12	59.76
C-9	54.14	47.57	44.80	44.65	47.45	53.99	49.25	47.01	46.57	47.66	47.18	46.48	46.28	47.71	47.48
C-10	38.07	37.87	37.23	36.32	36.75	37.98	37.81	37.94	37.87	37.94	37.87	38.10	38.13	36.89	38.33
C-11	18.38	18.78	18.04[c]	17.83[c]	17.95[c]	19.27	18.21	18.70	21.45	18.76	18.68	18.10[c]	18.06[c]	17.58	20.94
C-12	32.33	24.07	18.38[c]	18.32[c]	17.52[c]	28.22	33.14	24.68	31.15	24.13	24.68	17.79[c]	17.75[c]	31.32	31.21
C-13	42.32	43.31	38.13	39.56	43.29	41.29	43.45	40.26	81.84	43.28	39.62	43.46	39.79	81.97	81.89
C-14	38.52	42.69	42.26	42.16	42.40	37.37	37.52	42.72	49.32	42.71	42.80	42.55	42.60	49.21	49.19
C-15	41.16	128.22	130.12	130.34	128.95	40.60	45.00	131.19	127.72	128.66	128.84	128.94	129.33	128.48	128.23
C-16	153.01	145.06	143.89	143.53	144.77	66.00	153.77	143.29	145.57	145.26	148.84	144.82	148.52	145.21	145.47
C-17	105.05	15.42	15.54	15.44	15.32	49.96	104.26	62.00	11.66	15.42	60.86	15.45	61.01	11.64	11.66
C-18	67.29	67.14	70.70	72.15	71.95	66.15	68.23	67.10	67.13	66.15	65.96	70.87	70.60	71.98	64.54
C-19	16.10	16.00	17.84[d]	17.86[d]	16.84[d]	16.39	17.11	16.05	16.00	16.13	16.12	16.96[d]	16.97[d]	16.99[c]	12.49
C-20	17.26	17.20	17.75[d]	17.39[d]	16.79[d]	16.62	17.33	17.27	17.25	16.47	16.54	16.96[d]	16.93[d]	16.90[c]	17.22
*Me*CO		20.84		21.14	20.93		21.18	20.89	20.89					21.06	21.23
							20.85	20.89							20.99
Me*C*O		170.32		170.96	170.96		170.42	170.90	170.37					171.14	170.93
							170.24	170.38							170.40

[a]Values are from García-Granados *et al.* (4).

[b]These assignments were made with the aid of C/H correlation spectroscopy.

[c,d]Assignments in the same column with the same superscript may be interchanged.

temperature. After cc, 860 mg of *ent*-kaur-15-ene-7α,18-diol (sideridiol [**6**]) (13) was obtained. Product **6** (800 mg) was acetylated with pyridine-Ac_2O (50:20) for 2 h at 0°. After cc, 675 mg of *ent*-18-acetoxykaur-15-en-7α-ol (sideripol [**7**]) (14) was isolated: mp 83–85° (from hexane/$CHCl_3$); $[\alpha]_D$ +4° (*c* = 1, $CHCl_3$); ir ν max (KBr) cm^{-1} 3504, 1739, 1243, 1039; ^1H nmr (δ) 5.48 (1H, s, H-15), 4.00 and 3.44 (2H, AB system, J = 11.1 Hz, H_2-18), 3.54 (1H, dd, $J_1 = J_2$ = 2.8 Hz, H-7), 2.02 (3H, s, AcO), 1.67 (3H, d, J = 1.6 Hz, Me-17), 1.02 and 0.76 (3H each, s, Me-19 and Me-20); ^{13}C nmr see Table 1; ms *m/z* (%) $[M]^+$ 346 (14), 286 (7), 255 (11), 164 (16), 149 (9), 122 (26), 43 (100). Product **7** (600 mg) was dissolved in Me_2CO (25 ml) and oxidized with Jones reagent. After cc 490 mg of *ent*-18-acetoxykaur-15-en-7-one [**8**] was obtained as a gum: $[\alpha]_D$ −7° (*c* = 0.5, $CHCl_3$); ir ν max (neat) cm^{-1} 1739, 1700, 1380, 1336, 1238, 810, 755; ^1H nmr (δ) 5.44 (1H, s, H-15), 3.67 and 3.61 (2H, AB system, J = 11.4 Hz, H_2-18), 1.97 (3H, s, AcO), 1.67 (3H, d, J = 1.6 Hz, Me-17), 1.15 and 0.80 (3H each, Me-19 and Me-20); ^{13}C nmr see Table 1; ms *m/z* (%) $[M]^+$ 344 (100), 329 (7), 316 (15), 301 (20), 284 (15), 269 (56), 241 (25), 201 (13). Found C 76.3, H 9.5; $C_{22}H_{32}O_3$ requires C 76.70, H 9.36%.

ORGANISMS.—*Curvularia lunata* (strain CECT 2130) and *Rhizopus nigricans* (strain CECT 2672) used in these studies were from Colección Española de Cultivos Tipo, Departamento de Microbiología, Facultad de Ciencias, Universidad de Valencia, Spain.

MEDIA.—Medium YEPGA containing 1% yeast extract, 1% peptone, 2% glucose, and 2% agar, pH 5, was used for storage of microorganisms. In the transformation experiments a medium of the following composition was used: 0.1% peptone, 0.1% yeast extract, 0.1% beef extract, and 0.5% glucose in H_2O at pH 5.7.

CULTURE CONDITIONS.—Erlenmeyer flasks (250 ml) containing 60 ml of medium were inoculated with a dense suspension of microorganism in distilled H_2O prepared from slants. Incubations were maintained at 28° with shaking (150 rpm) for 6 days, after which the substrates in EtOH were distributed in the culture flasks and incubated as indicated.

RECOVERY AND PURIFICATION.—Cultures were filtered and pooled, and the cells were washed twice with H_2O. The liquid was saturated with NaCl and extracted repeatedly with CH_2Cl_2. These extracts were dried with $MgSO_4$ and evaporated at 40° in vacuo. The crude products were placed on a Si gel column (7 g of Si gel Merck 7729 was utilized for each 100 mg of mixture to separate) and eluted stepwise with CH_2Cl_2 containing increasing amounts of Me_2CO (to obtain a rapid gradient of polarity). Fractions that contained homogeneous material on tlc plates [Si gel 0.25 mm, Merck G, developed with CH_2Cl_2-Me_2CO (2:1)] were pooled. Starting material and products were detected with an H_2O-H_2SO_4-HOAc (3:1:16) spray, followed by heating at 120°.

BIOTRANSFORMATION OF *ENT*-18-ACETOXYKAUR-16-ENE-3,7-DIONE [**4**] BY *C. LUNATA*.—Substrate **4** (600 mg) in EtOH (10 ml) was distributed among ten culture flasks of *C. lunata* and incubated for 48 h after which, proceeding as indicated above, 90 mg of *ent*-18-hydroxykaur-16-ene-3,7-dione [**9**] (4) and 190 mg of *ent*-18-hydroxy-16β,17-epoxykaur-3,7-dione [**10**] were isolated. Another mixture of products (60 mg) was acetylated with pyridine-Ac_2O (2:1) for 12 h at room temperature. After cc, 6 mg of *ent*-7α,18-diacetoxykaur-16-en-3-one [**11**], 25 mg of *ent*-17,18-diacetoxy-16β-hydroxykaur-3,7-dione [**12**] (4), and 20 mg of *ent*-17,18-diacetoxy-16α-hydroxykaur-3,7-dione [**13**] (4) were isolated.

ENT-18-HYDROXY-16β,17-EPOXYKAUR-3,7-DIONE [**10**].—Mp 150–152° (from hexane/$CHCl_3$); $[\alpha]_D$ −30° (*c* = 1, $CHCl_3$); ir ν max (KBr) cm^{-1} 3443, 1702, 1059, 735; ^1H nmr (δ) 3.64 and 3.26 (2H, AB system, J = 10.3 Hz, H_2-18), 2.88 and 2.79 (2H, AB system, J = 4.5 Hz, H_2-17), 1.33 and 0.96 (3H each, s, Me-19 and Me-20); ^{13}C nmr see Table 1; ms *m/z* (%) $[M]^+$ 332 (2), 302 (37), 231 (7), 217 (8), 189 (20), 150 (36), 135 (66), 91 (93), 54 (100).

ENT-7α,18-DIACETOXYKAUR-16-EN-3-ONE- [**11**].—Gum; $[\alpha]_D$ −17° (*c* = 0.5, $CHCl_3$); ir ν max (neat) cm^{-1} 1738, 1706, 1460, 1374, 1244, 1039; ^1H nmr (δ) 4.82 (2H, dd, J_1 = 5.7, J_2 = 2.9, H-7 and H-17), 4.78 (1H, s, H-17), 4.02 and 3.91 (2H, AB system, J = 10.9 Hz, H_2-18), 2.02 and 2.00 (3H each, s, AcO), 1.10 and 0.99 (3H each, s, Me-19 and Me-20); ^{13}C nmr see Table 1; ms *m/z* (%) $[M]^+$ 402 (1), 342 (33), 282 (38), 267 (25), 198 (39), 149 (100), 105 (43), 91 (69).

ACETYLATION OF METABOLITE **9**.—Metabolite **9** (25 mg) was acetylated with pyridine-Ac_2O (1:0.5) for 2 h at room temperature. After cc, 21 mg of a product identical to substrate **4** was isolated.

ACETYLATION OF METABOLITE **10**.—Metabolite 10 (70 mg) was acetylated with pyridine-Ac_2O (2:1) for 2 h at room temperature. After cc, 60 mg of *ent*-18-acetoxy-16β,17-epoxykaur-3,7-dione [**14**] (1) was isolated.

BIOTRANSFORMATION OF *ENT*-18-ACETOXYKAUR-15-ENE-3,7-DIONE [**5**] BY *R. NIGRICANS*.—

Substrate **5** (150 mg) in EtOH (4 ml) was distributed among four culture flasks of *R. nigricans*. After 78 h of incubation, 36 mg of starting product **5** and 90 mg of a mixture of polar products were recovered. This last mixture was acetylated with pyridine-Ac_2O (4:2) for 12 h at room temperature. After cc, 29 mg of *ent*-17,18-diacetoxykaur-15-ene-3,7-dione [**15**] and 40 mg of *ent*-18-acetoxy-13-hydroxykaur-15-ene-3,7-dione [**16**] were isolated.

ENT-17,18-DIACETOXYKAUR-15-ENE-3,7-DIONE [**15**].—Gum; $[\alpha]_D$ −14° (c = 0.5, $CHCl_3$); ir ν max (neat) cm^{-1} 1742, 1705, 1236, 1039; ^1H nmr (δ) 5.86 (1H, bs, H-15), 4.69 and 4.62 (2H, part AB of a ABX system, J_{AB} = 14.1, J_{AX} = 1.7, J_{BX} = 1.5 Hz, H_2-17), 4.18 and 3.78 (2H, AB system, J = 11.3 Hz, H_2-18), 2.06 and 1.98 (3H each, s, AcO), 1.35 and 1.02 (3H each, s, Me-19 and Me-20); ^{13}C nmr see Table 1; ms *m/z* (%) $[M]^+$ 416 (10), 356 (22), 314 (21), 329 (10), 212 (9), 177 (15), 163 (10), 147 (21), 105 (80), 91 (100).

ENT-18-ACETOXY-13-HYDROXYKAUR-15-ENE-3,7-DIONE [**16**].—Gum; $[\alpha]_D$ −24° (c = 0.5, $CHCl_3$); ir ν max (neat) cm^{-1} 3467, 1744, 1706, 1237, 1039, 754; ^1H nmr (δ) 5.56 (1H, s, H-15), 4.18 and 3.77 (2H, AB system, J = 11.3 Hz, H_2-18), 1.98 (3H, s, AcO), 1.70 (3H, d, J = 1.6 Hz, Me-17), 1.34 and 1.02 (3H each, s, Me-19 and Me-20); ^{13}C nmr see Table 1; ms *m/z* (%) $[M]^+$ 374 (33), 314 (8), 197 (5), 178 (21), 163 (60), 136 (100), 91 (45).

BIOTRANSFORMATION OF *ENT*-18-ACETOXYKAUR-15-ENE-3,7-DIONE [**5**] BY *C. LUNATA*.—Substrate **5** (250 mg) in EtOH (4 ml) was distributed among four culture flasks of *C. lunata*. After 30 h of incubation 50 mg of *ent*-18-hydroxykaur-15-ene-3,7-dione [**17**] and 145 mg of *ent*-17,18-dihydroxykaur-15-ene-3,7-dione [**18**] were isolated.

ENT-18-HYDROXYKAUR-15-ENE-3,7-DIONE [**17**].—Mp 151–153° (from hexane/$CHCl_3$); $[\alpha]_D$ −51° (c = 1, $CHCl_3$); ir ν max (KBr) cm^{-1} 3442, 1693, 1062; ^1H nmr (δ) 5.47 (1H, s, H-15), 3.65 and 3.29 (2H, AB system, J = 11.3 Hz, H_2-18), 1.73 (3H, d, J = 1.5 Hz, Me-17), 1.37 and 0.97 (3H each, s, Me-19 and Me-20); ^{13}C nmr see Table 1; ms *m/z* (%) $[M]^+$ 316 (100), 301 (14), 286 (20), 275 (12), 243 (17), 201 (11), 159 (13).

ENT-17,18-DIHYDROXYKAUR-15-ENE-3,7-DIONE [**18**].—Mp 166–168° (from hexane/$CHCl_3$); $[\alpha]_D$ −51° (c = 1, EtOH); ir ν max (KBr), cm^{-1} 3339, 1691, 1070, 1021, 838; ^1H nmr (δ) 5.75 (1H, s, H-15), 4.19 (2H, collapsed AB system, H_2-17), 3.68 and 3.29 (2H, AB system, J = 11.3 Hz, H_2-18), 1.37 and 0.98 (3H each, s, Me-19 and Me-20); ^{13}C nmr see Table 1; ms *m/z* (%) $[M]^+$ 332 (19), 315 (14), 303 (100), 285 (55), 267 (4).

ACETYLATION OF METABOLITE **17**.—Metabolite **17** (20 mg) was acetylated with pyridine-Ac_2O (1:0.5) for 5 h at 0°. After cc, 15 mg of a product identical to substrate **5** was obtained.

ACETYLATION OF METABOLITE **18**.—Metabolite **18** (25 mg) was acetylated with pyridine-Ac_2O (1:0.5) for 5 h at 0°. After cc, 20 mg of product **15** was isolated.

BIOTRANSFORMATION OF *ENT*-18-ACETOXYKAUR-15-EN-7-ONE [**8**] BY *C. LUNATA*.—Substrate **8** (250 mg) in EtOH (4 ml) was distributed among four culture flasks of *C. lunata* and incubated for 48 h, after which 62 mg of starting product **8**, 26 mg of *ent*-18-hydroxykaur-15-en-7-one [**19**], and 45 mg of *ent*-17,18-dihydroxykaur-15-en-7-one [**20**] were isolated.

ENT-18-HYDROXYKAUR-15-EN-7-ONE [**19**].—Mp 103–105° (from hexane/$CHCl_3$); $[\alpha]_D$ −27° (c = 1, $CHCl_3$); ir ν max (KBr) cm^{-1} 3410, 1693, 1440, 1043, 810; ^1H nmr (δ) 5.47 (1H, s, H-15), 3.37 and 3.04 (2H, AB system, J = 11.4 Hz, H_2-18), 1.73 (3H, d, J = 1.4 Hz, Me-17), 1.20 and 0.77 (3H each, s, Me-19 and Me-20); ^{13}C nmr see Table 1; ms *m/z* (%) $[M]^+$ 302 (78), 287 (29), 269 (17), 259 (29), 229 (12), 201 (18).

ENT-17,18-DIHYDROXYKAUR-15-EN-7-ONE [**20**].—Mp 135–137° (from hexane/$CHCl_3$); $[\alpha]_D$ −27° (c = 1, $CHCl_3$); ir ν max (KBr) cm^{-1} 3376, 1600, 1322, 1068, 1016, 973, 834; ^1H nmr (δ) 5.76 (1H, s H-15), 4.18 (2H, collapsed AB system, H_2-17), 3.38 and 3.01 (2H, AB system, J = 11.4 Hz, H_2-18), 1.21 and 0.76 (3H each, s, Me-19 and Me-20); ^{13}C nmr see Table 1; ms *m/z* (%) $[M]^+$ 318 (20), 290 (18), 285 (15), 269 (17), 229 (6), 199 (8), 161 (4).

ACETYLATION OF METABOLITE **19**.—Metabolite **19** (10 mg) was acetylated with pyridine-Ac_2O (1:0.5) for 4 h at 0°. After cc, 7 mg of a product identical to substrate **8** was isolated.

BIOTRANSFORMATION OF *ENT*-18-ACETOXYKAUR-15-EN-7-ONE [**8**] BY *R. NIGRICANS*.—Substrate **8** (230 mg) in EtOH (10 ml) was distributed among ten culture flasks of *R. nigricans* and incubated for 92 h. After cc, 52 mg of starting product **8**, 64 mg of *ent*-18-acetoxy-13-hydroxykaur-15-en-7-one

[**21**] and 85 mg of a mixture of products were isolated. The mixture of products, after acetylation in pyridine-Ac_2O (3:1.5) for 12 h at room temperature, yielded 45 mg of *ent*-3β,18-diacetoxy-13-hydroxykaur-15-en-7-one [**22**].

ENT-18-ACETOXY-13-HYDROXYKAUR-15-EN-7-ONE [**21**].—Gum; $[\alpha]_D$ −19° (c = 0.5, $CHCl_3$); ir ν max (neat) cm^{-1} 3460, 1742, 1704, 1250, 1036; ^{1}H nmr (δ) 5.52 (1H, s, H-15), 3.72 and 3.65 (2H, AB system, J = 11.3 Hz, H_2-18), 2.04 (3H, s, AcO), 1.68 (3H, d, J = 1.5 Hz, Me-17), 1.20 and 0.85 (3H each, Me-19 and Me-20); ^{13}C nmr see Table 1; ms *m/z* (%) $[M]^+$ 360 (25), 345 (5), 343 (4), 300 (3), 282 (1), 259 (1), 257 (1), 229 (1).

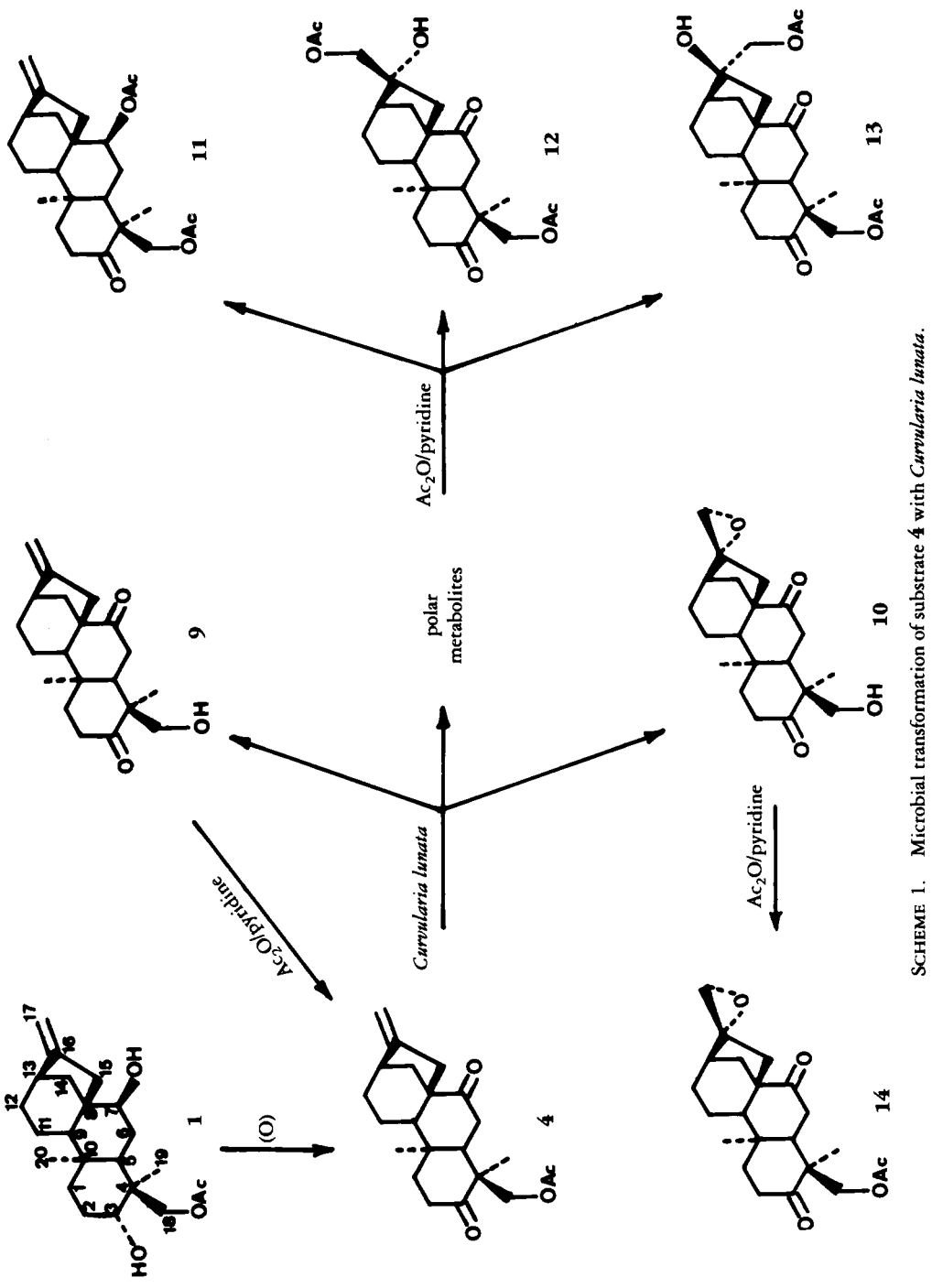
ENT-3β,18-DIACETOXY-13-HYDROXYKAUR-15-EN-7-ONE [**22**].—Gum; $[\alpha]_D$ −28° (c = 1, $CHCl_3$); ir ν max (neat) cm^{-1} 3488, 1740, 1705, 1244, 1040, 754; ^{1}H nmr (δ) 5.54 (1H, s, H-15), 4.73 (1H, dd, J_1 = 11.6 Hz, J_2 = 4.8, H-3), 3.85 and 3.56 (2H, AB system, J = 11.9, H_2-18), 2.03 and 2.02 (3H, s, AcO), 1.69 (3H, d, J = 1.6 Hz, Me-17), 1.24 and 0.86 (3H each, Me-19 and Me-20); ^{13}C nmr see Table 1; ms *m/z* (%) $[M]^+$ 418 (5), 401 (1), 376 (1), 359 (1), 343 (1), 314 (2), 298 (2), 265 (2), 255 (2), 241 (2).

RESULTS AND DISCUSSION

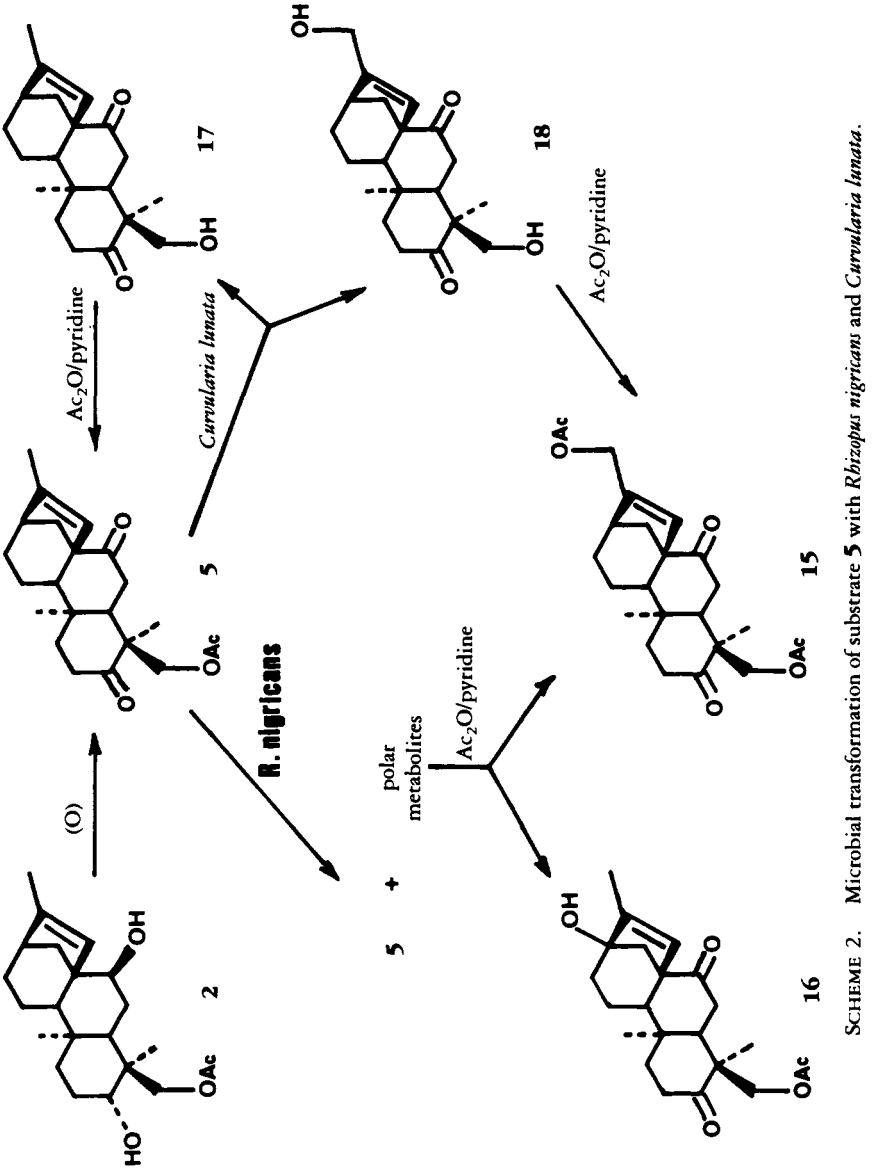
ent-18-Acetoxykaur-16-ene-3,7-dione [**4**] was incubated with *C. lunata* for 48 h, after which substrate **4** had been completely biotransformed by the fungus to give the deacetylated metabolite **9** (17%), the epoxyderivative **10** (34%) and a mixture of more polar products (Scheme 1). The structure of **9** was easily demonstrated by its acetylation to give substrate **4**. Metabolite **10** was acetylated to produce **14**, identical to another product isolated from the incubation of **4** with *R. nigricans* (1) (Table 1). The acetylation of the mixture of polar products obtained from this incubation gave products **11** (0.9% of substrate **4**), **12** (3.4%), and **13** (3%). The structure of **11** was determined principally by ^{13}C nmr (Table 1). Only one ketone group can be seen in this spectrum (δ 214.55). Hence one of the original ketone groups must have been reduced by the fungus and the resulting hydroxyl group then chemically acetylated. The study of the carbon chemical shifts 6, 7, 8, 9, and 15 [principally the γ effect on C-9 (15,16) and the coupling constants of the geminal protons to the new acetoxyl group (δ 4.82, 1H, dd, J_1 = 5.7, J_2 = 2.9 Hz)] led us to assign an axial disposition on C-7 for this new acetoxyl group. As *C. lunata* seems to deacetylate at C-18, the original metabolite may be *ent*-7α,18-dihydroxykaur-16-en-3-one. Products **12** and **13** turned out to be identical to the 16,17-glycol acetates which we described when we incubated substrate **4** with *Aspergillus niger* cultures (4).

The biotransformations carried out on *ent*-18-acetoxykaur-15-ene-3,7-dione [**5**] are described in Scheme 2. As can be seen, substrate **5** is the endo isomer of substrate **4**, which we have previously incubated with *R. nigricans* (1). *R. nigricans* partially biotransformed substrate **5** (24% of substrate **5** was recovered unaltered after 78 h of incubation). After acetylation of the mixture of metabolites, diacetate **15** (16%) and monoacetate **16** (25%) were isolated. The ms of **15** showed a molecular peak of *m/z* 416, indicating that **15** had a new acetoxyl group, which agrees with an ABX system in its ^{1}H-nmr spectrum. The part X is the olefin proton at C-15 (δ 5.86, bs) and the AB part is a double doublet (δ_A 4.69, J_1 = 14.1, J_2 = 1.7 Hz, δ_B 4.62, J_1 = 14.1, J_2 = 1.5 Hz). The ^{13}C-nmr spectrum of **15** confirms the presence of this new function at C-17 (Table 1). As *R. nigricans* usually does not alter the acetyl group at C-18, the only action of the fungus on substrate **5** probably is the hydroxylation at C-17.

Metabolite **16** had an ms with a molecular peak of *m/z* 374, which indicated that **5** had been hydroxylated by the fungus. Nevertheless, the ^{1}H-nmr spectrum of **16** indicated that the new hydroxyl group was introduced at a tertiary carbon site. The ^{13}C-nmr spectrum of **16** confirmed the presence of a new oxygenated carbon (δ 81.84, quaternary carbon). A study of the chemical shifts of the carbons 12, 13, 14, and 17 compared to those of substrate **5** allowed us to conclude that the new hydroxyl group



SCHEME 1. Microbial transformation of substrate **4** with *Curvularia lunata*.



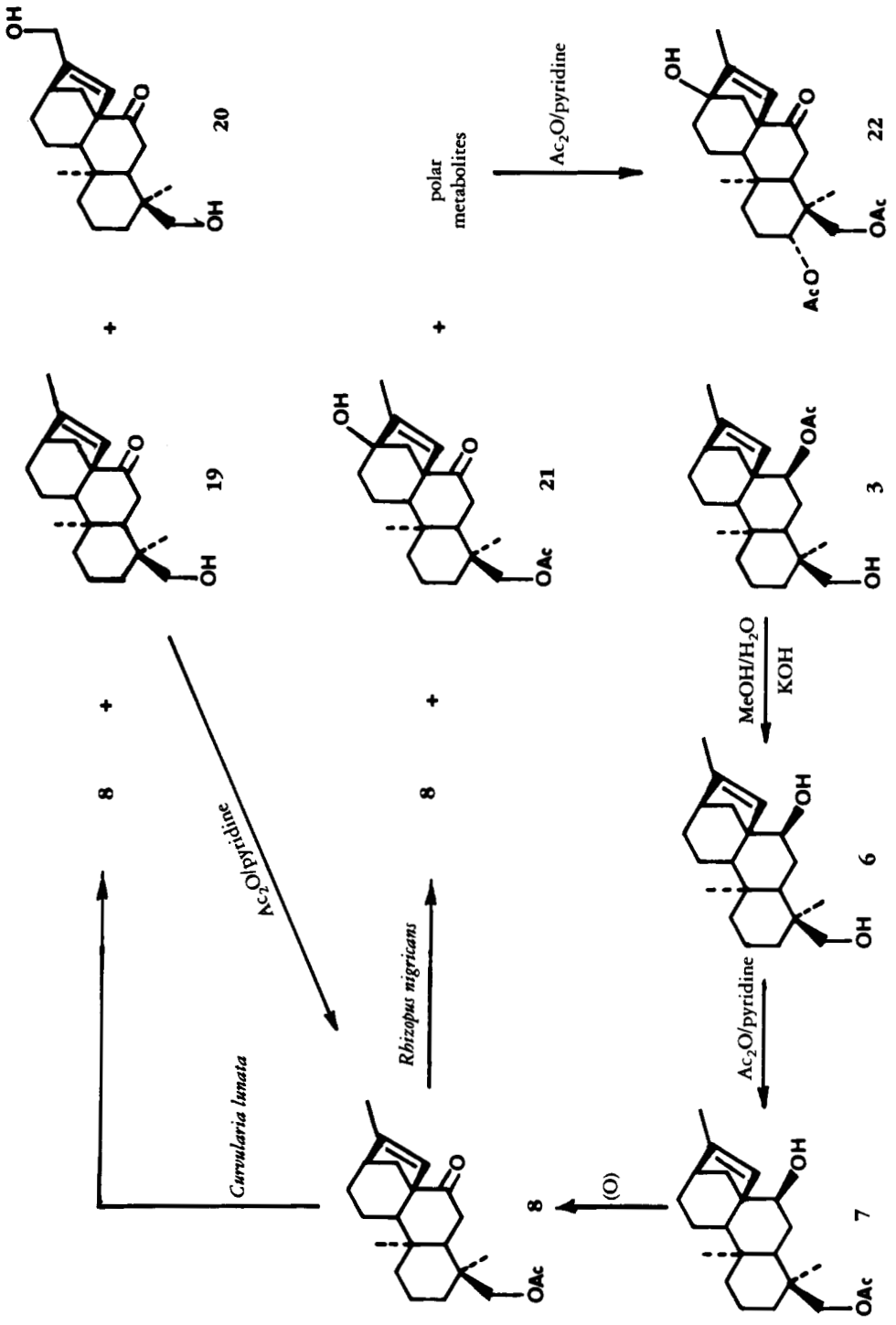
SCHEME 2. Microbial transformation of substrate **5** with *Rhizopus nigricans* and *Curvularia lunata*.

was introduced at C-13. Thus, the α effect on C-13 ($\Delta\delta = +38.53$), β effects on C-12 ($\Delta\delta = +7.08$) and C-14 ($\Delta\delta = +6.63$), and γ effect on C-17 ($\Delta\delta = -3.76$) are obvious.

The biotransformation of substrate **5** with *C. lunata* was complete after 30 h, and the deacetylated products **17** (23%) and **18** (62%) were isolated (Scheme 2). The acetylation of **17** gave **5**. Metabolite **18** showed a ^{1}H-nmr spectrum (collapsed AB system at δ 4.19 and AB system at δ 3.68 and 3.29, $J = 11.3$ Hz) and ^{13}C-nmr spectrum (Table 1) which led us to assign two hydroxyl groups on C-17 and C-18. This was confirmed by acetylation of **18** to give **15** described above.

Deacetylation at C-18 by *C. lunata* seens to be normal. Thus, after 48 h incubation of substrate **8** (Scheme 3) with this fungus, 25% of the substrate was recovered plus two nonacetylated products (**19**, 12% and **20**, 19%). The spectral data of **19** seemed to indicate that it might be the result of deacetylation at C-18 of substrate **8**. Acetylation of **19** confirmed this hypothesis. The ^{1}H-nmr spectrum of metabolite **20** showed two AB systems (δ 4.18, 2H, collapsed AB system, and δ 3.38 and 3.01, AB system, $J = 11.4$ Hz). On the other hand, no signal of the allylic methyl group could be observed; hence metabolite **20** might be a 17,18-dihydroxyderivative. The ^{13}C-nmr spectrum of **20** confirmed this proposed structure (Table 1). The new function at C-17 was confirmed by a comparison of the chemical shifts of substrate **8** and metabolite **20**. Moreover, the chemical shifts of the carbons of the rings C and D agreed with those described for similar carbons in the case of the previously discussed metabolite **18**. Nevertheless, incubation of **8** with *R. nigricans* cultures leaves the acetoxy group at C-18 unaltered. Thus, after 92 h incubation, as well as 23% unaltered **8**, metabolite **21** (27%) and a mixture of more polar products were isolated. The structure of metabolite **21** was deduced as follows. Its mass spectrum revealed that a new hydroxyl group was present in the molecule. Nevertheless, no proton geminal to the oxygenated function was detected in its ^{1}H-nmr spectrum. This is explicable on studying the ^{13}C-nmr spectrum of **21** because a new quaternary oxygenated carbon at δ 81.97 can be seen. This chemical shift and those assigned to C-12, C-14, and C-17, in accordance with the similar carbon of **16**, allowed us to confirm a new hydroxylation at C-13. Acetylation of the mixture of polar products gave product **22** (17% of **8**). The ^{1}H-nmr spectrum of **22** indicated that **22** had a primary and a secondary acetoxyl group. Nevertheless, methyl groups at C-17, C-19, and C-20 and the double bond were unaltered. The ^{13}C nmr of **22** showed signals of only one ketone group (δ 211.53) and three hydroxylated or acetoxylated carbons at δ 81.89 (quaternary), 73.61 (methine), and 64.54 (methylene). A study of all the chemical shifts of **22** indicated that a hydroxylation at C-13 had also occurred. A comparison of the chemical shifts of metabolite **22** with respect to those of **21** reveals another hydroxylation at C-3 of **22**. The mixture of polar products previously mentioned was the result of the equilibrium in the migration of the acetyl group between C-18 and C-3, as has been described elsewhere (1,2). Thus, *R. nigricans* hydroxylated substrate **8** at C-13 and probably then at C-3.

As illustrated in Figure 1 taking everything into account, including our previously published results (1,2,4), the action of the microorganisms *R. nigricans*, *C. lunata*, and *A. niger* on *ent*-kaur-16-enes functionalized the double bond to give *ent*-16β,17-epoxy compound (*C. lunata* and *R. nigricans*), *ent*-16α,17- and *ent*-16β,17-glycols (*C. lunata* and *A. niger*), and *ent*-16β-hydroxyl and *ent*-(16*S*),17-hydroxykaurane (*A. niger*). As far as the ketone group is concerned, no reduction was observed with *A. niger* cultures at C-3 and C-7 in *ent*-kaur-16-enes. Nevertheless, *R. nigricans* reduced at C-3 via the *ent*-β face and *C. lunata* reduced at C-7 also via the *ent*-β face. In the case of the *ent*-kaur-15-enes, the action of *C. lunata* is not dependent on the presence or absence of the ketone group at C-3 (except with regard to the yield of biotransformation). *C. lunata* hydroxyl-



SCHEME 3. Microbial transformation of substrate **8** with *Rhizopus nigricans* and *Curvularia lunata*.

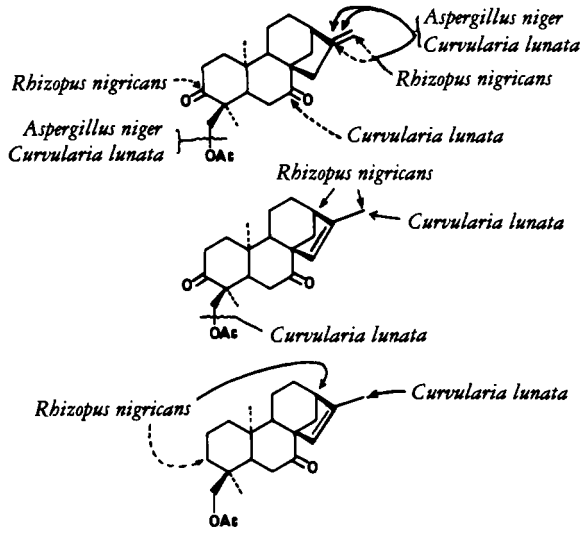


FIGURE 1. Summary of the action of *Rhizopus nigricans*, *Aspergillus niger*, and *Curvularia lunata* on *ent*-kaur-15-enones and *ent*-kaur-16-ene-3,7-dienones.

ated at C-17 of all the substrates. On the other hand *R. nigricans* tended to hydroxylate at C-13, and if the substrate had no ketone group at C-3 a presumably posterior *ent*-3β-hydroxylation of former metabolite occurred. If the substrate did have a ketone group at C-3, however, *R. nigricans* also hydroxylated at C-17, with a similar yield to the hydroxylation at C-13.

Finally, it should also be mentioned that *C. lunata* and *A. niger* normally produced desacetylation at C-18 while *R. nigricans* left the 18-acetate unaltered.

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