197. Microbial Metabolism of the Diterpene Sclareol: Oxidation of the A Ring by *Septomyxa affinis*

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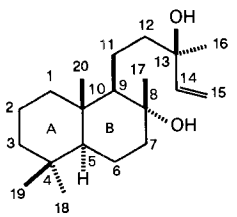
Microbial metabolism of the diterpene sclareol (**1**) was studied. Screening studies have shown a number of microorganisms capable of metabolizing **1**. Preparative-scale fermentation with *Septomyxa affinis* ATCC 6737 has resulted in the production of three fungal metabolites that have been characterized by 2D-NMR techniques and chemical reactions. These metabolites have been identified as 8α,13β-dihydroxylabd-14-en-3-one (**2**), labd-14-ene-3β,8α,13β-triol (**4**), and labd-14-ene-2α,8α,13β-triol (**6**).

Introduction. – Sclareol (**1**), a labdane diterpene ditertiary alcohol, is widely distributed in nature. It was first isolated from the essential oil of *Salvia sclarea* L. (Labiatae) in 1931 [1]. Sclareol is a fungal-growth regulator and a plant-growth inhibitor [2–4] and was reported to have a high antibacterial activity [5]. Commercially, sclareol is used as a fixative and natural body in perfumery, as a flavoring agent in the tobacco industry, and as a synthon for the preparation of a series of Ambra odorants in perfumery [6] [7]. The essential oil of *Salvia sclarea* L. (clary sage oil) is used extensively as a flavoring component in food products, wine essences, grape flavors and liqueurs, as a fragrance component in soaps, detergents, creams, lotions and tabac-type fragrances in perfumery, as a modifier in spice compounds as well as in folk medicine [8] [9].

Synthesis and the configuration at C(13) of sclareol were studied by *Bigley et al.* [10], and the (13*R*)-configuration has been confirmed [11]. The natural product **1** is a 9:1 epimeric mixture at C(13), the (13*R*)-epimer being the major component [6] [11]. Naturally occurring A-ring-oxygenated analogs of sclareol are unknown. Functionalization of



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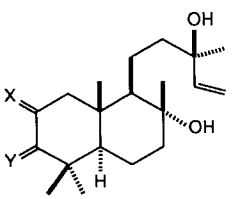
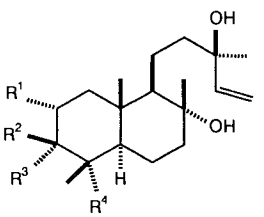


2 X = H_2, Y = O
3 X = O, Y = H_2



4 $R^1 = R^3 = H$, $R^2 = OH$, $R^4 = CH_3$
5 $R^1 = R^2 = R^3 = H$, $R^4 = CH_2OH$
6 $R^1 = OH$, $R^2 = R^3 = H$, $R^4 = CH_3$
7 $R^1 = R^3 = H$, $R^2 = OCOCH_3$, $R^4 = CH_3$
8 $R^1 = R^2 = H$, $R^3 = OH$, $R^4 = CH_3$
9 $R^1 = R^2 = H$, $R^3 = OCOCH_3$, $R^4 = CH_3$
10 $R^1 = OCOCH_3$, $R^2 = R^3 = H$, $R^4 = CH_3$

the A ring of sclareol by chemical reactions is difficult and very low yielding. Oxidation of sclareol by the '*Gif* system' [12] has afforded the 2-keto and 3-keto derivatives **3** and **2** in 2.5 and 0.7% yield, respectively.

Since there have been no reports on the mammalian metabolism of sclareol, a prospective approach was undertaken to study the mammalian metabolism of this diterpene utilizing microorganisms, particularly fungi, as *in vitro* models for prediction, comparison, and preparation of the mammalian metabolites of sclareol [13]. Microbial systems are also useful to prepare odorants and fragrances, flavoring and aroma-producing compounds from the parent diterpene sclareol, and as biocatalysts for conducting highly stereo- and regioselective as well as high-yielding transformations of sclareol to compounds that are not accessible or accessible with difficulty by chemical reactions and that could be useful as synthons in natural-products chemistry.

In an earlier study, we have reported [14] the isolation of two fungal metabolites of sclareol from a preparative-scale fermentation of growing cultures of *Cunninghamella* species NRRL 5695. These two metabolites were characterized as labd-14-ene-3β,8α,13β-triol (**4**) and labd-14-ene-8α,13β,18-triol (**5**). By using resting-cell suspensions of *C.* species NRRL 5695, the yield of these two metabolites was improved, and compounds **4** and **5** were obtained in 36 and 50% yield, respectively. In the present study, we report the isolation of three fungal metabolites of sclareol from a preparative-scale fermentation of growing cultures of *Septomyxa affinis* ATCC 6737. Based on the chemical and spectroscopic data, especially of 2D-NMR techniques, these three metabolites have been identified as 8α,13β-dihydroxylabd-14-en-3-one (**2**), labd-14-ene-3β,8α,13β-triol (**4**), and labd-14-ene-2α,8α,13β-triol (**6**). The isolation and structure elucidation of these metabolites are described herein.

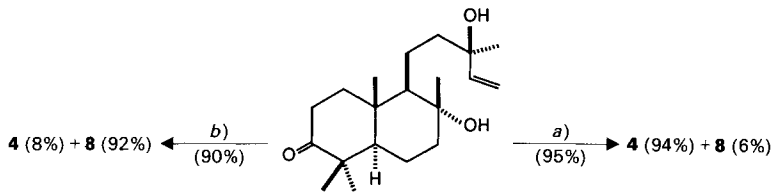
Results. – In an earlier study [14], a total of 45 microorganisms were screened for their ability to biotransform sclareol (**1**). In the present study, an additional 30 microorganisms were screened for the same purpose, and *Septomyxa affinis* ATCC 6737 was selected based on TLC analysis for preparative-scale fermentation of **1**. The ^{13}C-NMR chemical shift assignments of sclareol (**1**) have been reported unambiguously [12]. These assignments were further confirmed in our laboratory by a ^{13}C,^{13}C correlated 2D-INADEQUATE experiment [14].

A preparative-scale fermentation of **1** with *Septomyxa affinis* was performed, and three metabolites, **2**, **4**, and **6**, were isolated and purified by chromatography. The physical and spectral data of metabolite **4** were identical to that of 3β-hydroxysclareol (TLC, NMR, MS, IR, m.p., mixed m.p.), a fungal metabolite of sclareol that we have isolated earlier [14] from *Cunninghamella* species NRRL 5695. Compound **7**, the monoacetate of metabolite **4**, was also reported [14].

The MS of metabolite **2** ([$M - H_2O$]$^+$ at m/z 304) suggested that a single O-atom had been added to the substrate molecule, and that this O-atom is a ketonic functionality and not an OH group. This was supported by the ^{1}H-NMR spectrum of **2** (2 new *ddd* at 2.53 (J = 6.9, 11.7, 16.0 Hz) and 2.32 ppm (J = 3.4, 6.4, 16.0 Hz)), the DEPT GL experiment (disappearance of a CH_2 group), and the APT experiment (appearance of a new carbonyl C-atom at 216.6 ppm). Comparison of the ^{13}C-NMR spectral data of **1** and metabolite **2** suggested C(3) as the position of the keto function. This was further confirmed by chemical reactions which involved functional-group interconversions at C(3). Oxidation of the secondary alcohol **4** with CrO_3/pyridine afforded the 3-keto metabolite **2**, and

reduction of **2** with $NaBH_4$ in MeOH yielded the two expected isomers 3α-hydroxysclareol (**8**) and 3β-hydroxysclareol (**4**) in a 6:94 ratio, while reducing the ketone with $NaBH_4$ in i-PrOH afforded **8** and **4** in a 9:91 ratio (*Scheme*). The best yield of the 3α-isomer **8** was obtained on reduction of **2** with *K-Selectride*® at −78° (**8**/**4** 92:8). Compound **8** was also acetylated to give monoacetate **9**. ^{13}C- and ^1H-NMR chemical-shift assignments of compounds **2**, **8**, and **9** were based on $^1H,^1H$ and $^1H,^{13}C$ chemical shift correlated 2D-NMR spectroscopy which confirmed the proposed structures.

Scheme



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a) $NaBH_4$, MeOH, 0°. *b*) *K-Selectride*®, THF, −78°.

The MS data of metabolite **6** ($[M - H_2O]^+$ at *m/z* 306) suggested that a single O-atom had been added to the substrate molecule. The ^1H-NMR spectrum of **6** (new *tt* (J = 4.0, 11.3 Hz) at 3.82 ppm) and the DEPT GL experiment (disappearance of a CH_2 group and appearance of a new CH group at 64.5 ppm) indicated that metabolite **6** is a monohydroxylated (secondary) metabolite of sclareol. Comparison of the ^{13}C-NMR spectral data of **1** and metabolite **6** established C(2) as the position of hydroxylation. The configuration of the new OH group at C(2) was determined as being α using the ^1H-NMR data (H–C(2) is coupled with 2 H–C(3) and 2 H–C(1) with J = 4.0 and 11.3 Hz and, therefore, must be axial). This was further confirmed by acetylation of **6** to monoacetate **10**. Oxidation of the secondary alcohol **6** with CrO_3/pyridine afforded 2-keto derivative **3**. ^{13}C-NMR and ^1H-NMR chemical-shift assignments of metabolite **6**, its monoacetate **10**, and compound **3** were based on $^1H,^1H$ and $^1H,^{13}C$ chemical shift correlated 2D-NMR data which confirmed the proposed structures. ^{13}C-NMR chemical-shift assignments of C(1) and C(3) of compound **3** were based on a long-range HETCOR (LR HETCOR) experiment, with the J value optimized to 10 Hz in order to detect H,C 3-bond couplings; it revealed couplings between C(3) and H–C(18) and C(3) and H–C(19).

Discussion. – Microbial transformation reactions are known to be very useful biocatalysts in synthetic organic chemistry [15] [16]. They are all mediated by enzymes which are chiral catalysts with rigid substrate-binding characteristics. Thus, high degrees of regio- and stereoselectivities occur under extremely mild reaction conditions. Highly selective enzymatic transformations may occur with polyfunctional substrates without the need for protecting groups normally used in synthetic organic chemistry. Furthermore, microbial metabolites can be produced in relatively large quantities by microorganisms utilizing routine fermentation optimization and scaleup techniques or resting-cell suspensions of microbial cells. Useful microbial transformations may be conducted on very large industrial scale as well.

Functionalization of the nonactivated C-atoms of the A ring of sclareol is difficult to obtain by chemical reactions: oxidation of sclareol by the '*Gif* system' [12] afforded **2** and **3** in extremely low yields. Contrarily, the microbial transformation of sclareol (**1**) by *Septomyxa affinis* ATCC 6737 produced 2α-hydroxysclareol (**6**), 3β-hydroxysclareol (**4**), and the corresponding 3-ketone **2** in 27, 3, and 49% yield, respectively. Oxidation of **6** gave access to the 2-keto derivative **3** in 90% yield (24% yield from **1**). On the other hand, microbial transformations of sclareol (**1**) utilizing resting-cell suspensions of *Cunninghamella* species NRRL 5695 afforded 3β-hydroxysclareol (**4**) and 18-hydroxysclareol (**5**) in 36 and 50% yield, respectively [14]. These microbial systems are not only useful to obtain difficult-to-synthesize analogs of **1** in good yield, but also are useful as *in vitro* models to mimic and predict the mammalian metabolism of **1**. Further microbial metabolism studies of the diterpene **1** are in progress.

The authors wish to thank Dr. *B.M. Lawrence, R.J. Reynolds Tobacco Company,* Flavor Technology Division, for a generous supply of sclareol. We also acknowledge the Research Institute of Pharmaceutical Sciences of the University of Mississippi for financial support and the *Rho Chi* National Pharmacy Honor Society for a *Rho Chi* Graduate Scholarship to *S.A. Kouzi.*

Experimental Part

General. CrO_3, 4-(dimethylamino)pyridine, $NaBH_4$, and *K-Selectride*® (= 1.0M potassium tri(*sec*-butyl)-borohydride in THF) were obtained from *Aldrich Chemical Company,* Milwaukee, Wisconsin, and Ac_2O was purchased from *Mallinckrodt.* THF was distilled over Na with benzophenone as indicator. Pyridine, DMF, and CH_2Cl_2 were distilled from CaH_2 and stored over 4 Å molecular sieves. TLC: precoated silica *G-25 UV$_{254}$* plates (*Macherey-Nagel,* Düren); elution with AcOEt/hexane 9:1; detection by spraying with anisaldehyde/H_2SO_4 and then heating at 110° for 3 min. Flash CC: silica gel *60,* 230–400 mesh (*E. Merck,* Darmstadt). Gas chromatography: *Hewlett-Packard-5890A* gas chromatograph equipped with a flame ionization detector; *DB-5* 30 m × 0.25 mm capillary column, at 280° isothermal; He (1 ml/min) as carrier gas. M.p.: *Fisher* digital melting point analyzer, model *355,* or *Thomas-Hoover* capillary melting point apparatus (open capillary tubes), uncorrected. Optical rotations: *JASCO DIP-370* digital polarimeter. IR spectra (KBr; cm^{-1}): *Perkin-Elmer-281B* IR spectrophotometer. ^1H- and ^{13}C-NMR spectra ($CDCl_3$): *Varian-VXR-300* FT spectrometer, 300 and 75 MHz, resp.; δ in ppm, J in Hz; standard pulse sequences were used for COSY [17], HETCOR [18], DEPT GL [19], APT [20], and 2D-INADEQUATE [21] experiments. EI-MS (*m/z* (%)): *Finnigan-3200* mass spectrometer (70 eV ionization potential) coupled to a *Teknivent Vector/One* data system. HR-MS data were obtained at the MS Laboratory, Department of Chemistry, The University of Kansas, Lawrence, KS 66045.

Microorganisms and Media. The cultures were obtained from the University of Mississippi, Department of Pharmacognosy Culture Collection and were originally from the American Type Culture Collection (ATCC), Rockville, Maryland, or from Northern Regional Research Laboratories (NRRL), Peoria, Illinois. UI cultures were obtained from Dr. *John P. Rosazza,* Department of Medicinal Chemistry and Natural Products, College of Pharmacy, University of Iowa, Iowa City, Iowa 52242. Cultures used for preliminary screening of sclareol which showed one or more metabolites by TLC are as follows: *Aspergillus alliaceus* NRRL 315, *Curvularia lunata* ATCC 12017, *Coniophora puteana* ATCC 12675, *Fomes pinicola* ATCC 15341, *Mortierella zonata* ATCC 13309, *Septomyxa affinis* ATCC 6737, and *Trichophyton tonsurans* ATCC 10217. All the preliminary screening and scaleup experiments were carried out in a medium consisting of dextrose (20 g), yeast extract (5 g), peptone (5 g), NaCl (5 g), K_2HPO_4 (5 g), and dist. H_2O (1000 ml). Stock cultures of fungi and bacteria were stored on slants of *Mycophil* (*BBL,* Cockeysville, MD) and *Eugon* (*Difco,* Detroit, MI) agar, resp., at 4°.

Fermentation Procedures. Microbial metabolism studies were carried out by incubating the cultures with shaking on the model *G-10 Gyrotory* shaker (*New Brunswick Scientific Co.,* New Jersey), operating at 250 rpm at 25°. Preliminary screening experiments were carried out in 125-ml stainless-steel-capped *DeLong* culture flasks containing 25 ml of medium. The media were sterilized at 121°/18 psi for 15 min. Fermentations were carried out according to a standard two-stage protocol. In general, the substrate was added to the incubation media 24 h after the inoculation of the stage-II cultures as a 10% soln. in EtOH or DMF at a concentration of 0.2 mg/ml of stage-II medium. Substrate controls were composed of sterile medium to which the substrate was added and incubated

without microorganisms. Culture controls consisted of fermentation blanks in which the microorganisms were grown under identical conditions but without the substrate addition. Substrate-autoclaved-culture controls consisted of microbial cultures that were grown under the ususal conditions to maturity (usually 5–7 days), autoclaved for 30 min, and then incubated after the substrate was added.

Sclareol (**1**) was obtained as an off-white to amber-colored solid resin from *R. J. Reynolds Tobacco Company*, Flavor Technology Division, produced by the hydrocarbon solvent extraction of the plant *S. sclarea*. Flash CC (silica gel, AcOEt/hexane 1:4) and recrystallization of the homogeneous fractions from hexane yielded pure **1** (by TLC and GLC). White needles (85% yield from the resin). R_f 0.62. M.p. 96–97° ([6]: 99–100°). $[\alpha]_D^{25} = -2.82$ ($c = 1.45$, $CHCl_3$); [6]: $[\alpha]_D^{20} = -2.17$ ($c = 1.45$, $CHCl_3$). IR (KBr): 3280, 2910, 1635, 1450, 1380, 1360, 985, 910, 890. ^{1}H-NMR: 5.91 (*dd*, $J_{cis} = 10.5$, $J_{trans} = 17.2$, H–C(14)); 5.19 (*dd*, $J_{gem} = 1.2$, $J_{trans} = 17.2$, H–C(15) *trans* to H–C(14)); 4.98 (*dd*, $J_{gem} = 1.2$, $J_{cis} = 10.5$, H–C(15) *cis* to H–C(14)); 2.88 (br. *s*, exchangeable with D_2O, OH); 2.24 (br. *s*, exchangeable with D_2O, OH); 1.79 (*dt*, $J = 3.0, 12.1$, H–C(7)); 1.45–1.60 (*m*, 2H–C(12), H–C(1), H–C(6)); 1.39 (*m*, H–C(11), H–C(2)); 1.36 (*m*, H–C(7)); 1.32 (*dt*, $J = 3.5, 12.2$, H–C(3)); 1.26 (*m*, H–C(11)); 1.21 (*s*, CH_3(16)); 1.14 (*m*, H–C(3)); 1.11 (*s*, CH_3(17)); 1.04 (*t*, $J = 3.4$, H–C(9)); 0.86 (*dd*, $J = 2.4, 12.0$, H–C(5)); 0.81 (*s*, CH_3(18)); 0.74 (*s*, CH_3(19), CH_3(20)). ^{13}C-NMR: *Table*. EI-MS: 290 (6, $[M - H_2O]^+$), 275 (3), 272 (3), 257 (5), 203 (16), 195 (18), 191 (37), 177 (100), 163 (23), 149 (26), 137 (38), 121 (40), 109 (69), 95 (91), 81 (96), 71 (88).

Table. *^{13}C-NMR Chemical-Shift Assignments*[a]) *of Compounds* **1–4** *and* **6–10**[b])

	1	**2**	**3**	**4**	**6**	**7**	**8**	**9**	**10**
C(1)	39.5 (2)	37.9 (2)	55.1 (2)	37.8 (2)	48.4 (2)	37.3 (2)	32.5 (2)	33.1 (2)	44.5 (2)[e])
C(2)	18.3 (2)	33.6 (2)	211.6 (0)	27.1 (2)	64.5 (1)	23.2 (2)	25.1 (2)	22.6 (2)	68.6 (1)
C(3)	41.9 (2)	216.6 (0)	56.2 (2)	78.7 (1)	50.8 (2)	80.6 (1)	75.9 (1)	77.9 (1)	46.7 (2)
C(4)	33.1 (0)	47.1 (0)	38.6 (0)	38.8 (0)[c])	34.5 (0)	37.5 (0)	37.5 (0)	36.6 (0)	34.5 (0)
C(5)	55.9 (1)	54.7 (1)	55.2 (1)	54.9 (1)	55.4 (1)	54.9 (1)	48.8 (1)	49.8 (1)	55.4 (1)
C(6)	20.3 (2)	20.9 (2)	20.4 (2)	20.1 (2)	19.9 (2)	19.8 (2)	20.2 (2)	19.9 (2)	19.9 (2)
C(7)	43.9 (2)	43.0 (2)	43.1 (2)	44.1 (2)	43.7 (2)	43.6 (2)	44.3 (2)	44.0 (2)	43.6 (2)
C(8)	74.6 (0)	73.8 (0)	74.1 (0)	74.5 (0)	74.3 (0)	74.1 (0)	74.6 (0)	74.6 (0)	74.4 (0)
C(9)	61.6 (1)	60.2 (1)	60.7 (1)	61.4 (1)	61.3 (1)	61.2 (1)	61.4 (1)	61.2 (1)	61.3 (1)
C(10)	39.1 (0)	38.4 (0)	44.1 (0)	38.9 (0)[c])	40.5 (0)	38.6 (0)	39.0 (0)	38.9 (0)	40.5 (0)
C(11)	18.8 (2)	18.9 (2)	18.7 (2)	19.1 (2)	18.9 (2)	18.8 (2)	19.0 (2)	19.0 (2)	18.9 (2)
C(12)	44.8 (2)	44.3 (2)	44.4 (2)	44.8 (2)	44.7 (2)	44.6 (2)	44.9 (2)	44.8 (2)	44.4 (2)[e])
C(13)	73.2 (0)	73.1 (0)	73.2 (0)	73.6 (0)	73.3 (0)	73.1 (0)	73.6 (0)	73.5 (0)	73.4 (0)
C(14)	146.5 (1)	145.7 (1)	146.1 (1)	145.9 (1)	146.1 (1)	146.2 (1)	145.9 (1)	145.9 (1)	146.1 (1)
C(15)	110.7 (2)	110.9 (2)	110.9 (2)	111.2 (2)	110.9 (2)	110.7 (2)	111.2 (2)	111.2 (2)	110.9 (2)
C(16)	26.2 (3)	26.5 (3)	26.3 (3)	27.2 (3)	26.5 (3)	26.3 (3)	27.2 (3)	27.0 (3)	26.4 (3)
C(17)	23.9 (3)	23.5 (3)	23.6 (3)	24.1 (3)	24.0 (3)	23.8 (3)	24.1 (3)	24.2 (3)	24.1 (3)
C(18)	33.3 (3)	25.9 (3)	33.3 (3)	28.1 (3)	33.4 (3)	27.9 (3)	28.3 (3)	27.8 (3)	33.3 (3)
C(19)	21.4 (3)	21.0 (3)	22.9 (3)	15.4 (3)[d])	22.2 (3)	16.2 (3)	21.9 (3)	21.6 (3)	22.1 (3)
C(20)	15.3 (3)	14.5 (3)	16.4 (3)	15.3 (3)[d])	16.3 (3)	15.4 (3)	15.3 (3)	15.2 (3)	16.1 (3)
C(21)						170.8 (0)		170.7 (0)	170.6 (0)
C(22)						21.1 (3)		21.3 (3)	21.4 (3)

[a]) Assignments are based on ^{1}H,^{1}H and ^{1}H,^{13}C chemical-shift-correlated 2D-NMR spectroscopy and comparisons with the assignments of compound **1**. The assignments for **1** are based on a ^{13}C,^{13}C-correlated 2D-INADEQUATE experiment.

[b]) The number in parentheses indicates the number of H-atoms attached to the corresponding C-atom and was determined from DEPT GL experiments.

[c])[d])[e]) Assignments may be reversed.

Fungal Metabolism of Sclareol (**1**) *to 8α,13β-Dihydroxylabd-14-en-3-one* (**2**), *Labd-14-ene-3β,8α,13β-triol* (**4**), *and Labd-14-ene-2α,8α,13β-triol* (**6**). A soln. of 620 mg of sclareol (**1**) in 2 ml of DMF was distributed equally among 16 1-l culture flasks each containing 200 ml of 24-h-old *Septomyxa affinis* ATCC 6737 stage-II culture. After 7 days, the incubation mixtures were combined and filtered and the cells washed with dist. H_2O. The combined aq. filtrate (3.5 l) was extracted with 4 × 900 ml of AcOEt, the org. layer dried (Na_2SO_4) and evaporated, and the brownish-yellow residue (1.2 g) chromatographed (silica gel (120 g; 65 × 4 cm), AcOEt/hexane, 10-ml

fractions). Elution with AcOEt/hexane 1:4, gave 90 mg (14%) of **1** and elution with AcOEt/hexane 1:3, 335 mg of **2** as an oil. R_f 0.50. Crystallization from AcOEt/hexane afforded white needles of **2** (320 mg, 49%). M.p. 88–89°. $[\alpha]_D^{25} = -2.0$ ($c = 0.15$, $CHCl_3$); [12]: $[\alpha]_D^{22} = -2.5$ ($c = 0.81$, $CHCl_3$). IR: 3260, 2940, 2860, 1710, 1450, 1390, 1130, 1080, 1000, 930, 890. ^{1}H-NMR: 5.86 (*dd*, $J_{cis} = 10.7$, $J_{trans} = 17.3$, H–C(14)); 5.15 (*dd*, $J_{gem} = 1.4$, $J_{trans} = 17.3$, H–C(15) *trans* to H–C(14)); 4.95 (*dd*, $J_{gem} = 1.4$, $J_{cis} = 10.7$, H–C(15) *cis* to H–C(14)); 2.93 (br. *s*, exchangeable with D_2O, 2OH); 2.53 (*ddd*, $J = 6.9$, 11.7, 16.0, H–C(2)); 2.32 (*ddd*, $J = 3.4$, 6.4, 16.0, H–C(2)); 1.81–1.86 (*m*, H–C(7), H–C(1)); 1.50–1.56 (*m*, H–C(6), H–C(11)); 1.35–1.47 (*m*, H–C(5), H–C(7), H–C(1)); 1.30 (*m*, H–C(6)); 1.21 (*s*, CH_3(16)); 1.16 (*s*, CH_3(17)); 1.13 (*t*, $J = 3.2$, H–C(9)); 1.04 (*s*, CH_3(18)); 0.96 (*s*, CH_3(19)); 0.89 (*s*, CH_3(20)). ^{13}C-NMR: *Table*. EI-MS: 304 (4, $[M - H_2O]^+$), 289 (6), 286 (2), 271 (2), 209 (15), 206 (14), 191 (20), 121 (36), 109 (48), 107 (42), 95 (46), 93 (42), 83 (50), 81 (67), 71 (100), 69 (49), 67 (51), 55 (62). HR-MS: 304.2394 ($C_{20}H_{32}O_2$, $[M - H_2O]^+$, calc. 304.2401).

Elution with AcOEt/hexane 3:7 gave 29 mg of **4**. R_f 0.45. Recrystallization from AcOEt yielded white needles of **4** (20 mg, 3%). M.p. 163–164° ([14]: 162–163°). $[\alpha]_D^{25} = -7.5$ ($c = 0.15$, $CHCl_3$). IR: 3400, 2980, 2960, 2930, 2860, 1455, 1385, 1050, 995, 920. ^{1}H-NMR: 5.91 (*dd*, $J_{cis} = 10.7$, $J_{trans} = 17.3$, H–C(14)); 5.20 (*dd*, $J_{gem} = 1.4$, $J_{trans} = 17.3$, H–C(15) *trans* to H–C(14)); 5.02 (*dd*, $J_{gem} = 1.4$, $J_{cis} = 10.7$, H–C(15) *cis* to H–C(14)); 3.21 (*dd*, $J = 4.5$, 11.0, H–C(3)); 1.95 (br. *s*, exchangeable with D_2O, 2OH); 1.83 (*m*, H–C(7), H–C(12)); 1.55–1.67 (*m*, 2H–C(6), 2H–C(2), H–C(1), H–C(12)); 1.39 (*m*, H–C(7)); 1.26 (*s*, CH_3(16)); 1.15 (*s*, CH_3(17)); 1.10 (*t*, $J = 3.5$, HC(9)); 0.97 (*s*, CH_3(18)); 0.89 (*dd*, $J = 2.2$, 11.5 Hz, H–C(5)); 0.79 (*s*, CH_3(20)); 0.75 (*s*, CH_3(19)). ^{13}C-NMR: *Table*. EI-MS: 306 (9, $[M - H_2O]^+$), 291 (13), 288 (4), 273 (3), 255 (4), 237 (3), 235 (5), 223 (6), 209 (22), 207 (17), 194 (26), 190 (24), 175 (64), 109 (46), 81 (50), 71 (100). HR-MS: 306.2546 ($C_{20}H_{34}O_2$, $[M - H_2O]^+$, calc. 306.2557).

Elution with AcOEt/hexane 2:3 gave 200 mg of **6**. R_f 0.38. Purification by CC (70 × 2 cm, *Florisil*® (20 g, 200/300 A mesh, *Floridin Company*, Berkeley Springs, W. VA.), AcOEt/hexane/MeOH 20:80:0.1) and crystallization from AcOEt/hexane yielded white needles of **6** (175 mg, 27%). M.p. 89–90°. $[\alpha]_D^{25} = -22.0$ ($c = 0.05$, $CHCl_3$). IR: 3370, 2930, 2860, 1455, 1390, 1160, 1040, 910. ^{1}H-NMR: 5.85 (*dd*, $J_{cis} = 10.7$, $J_{trans} = 17.3$, H–C(14)); 5.14 (*dd*, $J_{gem} = 1.4$, $J_{trans} = 17.3$, H–C(15) *trans* to H–C(14)); 4.93 (*dd*, $J_{gem} = 1.4$, $J_{cis} = 10.7$, H–C(15) *cis* to H–C(14)); 3.82 (*tt*, $J = 4.0$, 11.3, H–C(2)); 3.02 (br. *s*, exchangeable with D_2O, 3OH); 1.95 (*dd*, $J = 2.6$, 12.9, H–C(1)); 1.79 (*dt*, $J = 3.1$, 12.0, H–C(7)); 1.69 (*dd*, $J = 3.0$, 12.5, H–C(3)); 1.55 (*m*, H–C(12), H–C(6)); 1.34 (*m*, H–C(7)); 1.21 (*s*, CH_3(16)); 1.09 (*s*, CH_3(17)); 0.86 (*s*, CH_3(18)); 0.83 (*m*, H–C(5), H–C(1)); 0.76 (*s*, CH_3(19), CH_3(20)). ^{13}C-NMR: *Table*. EI-MS: 306 (13, $[M - H_2O]^+$), 291 (6), 288 (9), 273 (7), 255 (7), 235 (5), 217 (6), 207 (9), 190 (24), 175 (68), 161 (21), 147 (34), 135 (44), 121 (43), 109 (52), 95 (65), 81 (67), 71 (100), 55 (54), 43 (95). HR-MS: 306.2546 ($C_{20}H_{34}O_2$, $[M - H_2O]^+$, calc. 306.2557).

Oxidation of **4** *to* **2**. CrO_3 (92.4 mg, 0.924 mmol) was added to a magnetically stirred soln. of 0.146 g (1.848 mmol) of dry pyridine in 10 ml of dry CH_2Cl_2 and the deep burgandy soln. was stirred for 15 min at r.t. (drying tube containing *Drierite*®). Then, 50 mg (0.154 mmol) of **4** in 2 ml of dry pyridine were added in one portion, and a tarry, black deposit started to separate. After 40 min stirring at r.t., the soln. was decanted from the residue which was washed with 10 ml of Et_2O. The combined org. phase was washed with 5% aq. NaOH, 5% aq. HCl, 5% aq. $NaHCO_3$, and sat. aq. NaCl soln., dried (Na_2SO_4), and evaporated: 55 mg of brownish residue. Purification by CC (52 × 1.5 cm), silica gel (3 g), AcOEt/hexane 1:3 and crystallization from AcOEt/hexane yielded 41 mg (82%) of **2** (m.p. 87–88°) which was identical in all respects (TLC, IR, NMR, MS, mixed m.p.) to metabolite **2**.

Reduction of Metabolite **2** *with* $NaBH_4$ *to* **4** *and Labd-14-ene-3α,8α,13β-triol* (**8**). A soln. of 375 mg (1.164 mmol) of **2** in 25 ml of MeOH was magnetically stirred at 0° in an ice-bath. Then, 66.08 mg (1.746 mmol) of $NaBH_4$ were added slowly. After 1 h stirring at 0°, H_2O/ice was added and the mixture extracted with AcOEt. The AcOEt extract was washed with H_2O, dried (Na_2SO_4), and evaporated: 370 mg of two products (TLC). The residue was chromatographed (silica gel (27 g; 64 × 3 cm), AcOEt/hexane). Elution with AcOEt/hexane 3:7 and recrystallization of the homogeneous fractions (R_f 0.45) from AcOEt yielded 340 mg (90%) of **4**, and elution with AcOEt/hexane 2:3 and recrystallization of the homogeneous fractions (R_f 0.31) from AcOEt/hexane yielded 20 mg (5%) of **8** as white needles. **8**: M.p. 110–111°. $[\alpha]_D^{25} = -23.0$ ($c = 0.1$, $CHCl_3$). IR: 3360, 2940, 2870, 1460, 1390, 1140, 1075, 995, 945, 925. ^{1}H-NMR: 5.92 (*dd*, $J_{cis} = 10.7$, $J_{trans} = 17.3$, H–C(14)); 5.20 (*dd*, $J_{gem} = 1.4$, $J_{trans} = 17.3$, H–C(15) *trans* to H–C(14)); 5.01 (*dd*, $J_{gem} = 1.4$, $J_{cis} = 10.7$, H–C(15) *cis* to H–C(14)); 3.39 (*dd*, $J = 2.7$, 2.7, H–C(3)); 1.82–1.94 (*m*, H–C(7), H–C(2)); 1.66 (*m*, H–C(12)); 1.49–1.61 (*m*, H–C(2), H–C(6)); 1.36–1.47 (*m*, H–C(5), H–C(7), H–C(1)); 1.26 (*s*, CH_3(16)); 1.21 (*t*, $J = 3.7$, H–C(9)); 1.14 (*s*, CH_3(17)); 0.94 (*s*, CH_3(18)); 0.81 (*s*, CH_3(19)); 0.79 (*s*, CH_3(20)). ^{13}C-NMR: *Table*. EI-MS: 306 (10, $[M - H_2O]^+$), 291 (7), 288 (7), 273 (5), 255 (7), 235 (6), 223 (6), 207 (12), 190 (37), 175 (100), 161 (32), 147 (41), 135 (86), 121 (49), 109 (50), 95 (46), 81 (52). HR-MS: 306.2546 ($C_{20}H_{34}O_2$, $[M - H_2O]^+$, calc. 306.2557).

Reduction of Metabolite **2** *by* K-Selectride® *to* **4** *and* **8**. To potassium tri(*sec*-butyl)borohydride (2.142 ml of a 1.0M soln., 2.142 mmol) in THF in a dry flask under N_2 at −78°, a soln. of 345 mg (1.071 mmol) of **2** in 7 ml of dry

THF was added dropwise. The mixture was stirred at −78° for 3 h and at 0° for 1 h and then warmed up to r.t. The reaction was quenched with 3 ml of 4M NaOH followed by 5 ml of 30% H_2O_2 soln. After stirring for 0.5 h at r.t. (exothermic), the aq. layer was extracted with Et_2O, the org. layer washed with sat. aq. NaCl soln., dried (Na_2SO_4), and evaporated, and the residue (335 mg) separated by flash CC (silica gel (25 g; 64 × 3 cm), AcOEt/hexane). Recrystallization of the homogeneous fractions (R_f 0.45 and 0.31) from AcOEt/hexane yielded 4 (24 mg, 7%) and **8** (289 mg, 83%), resp.

8α,13β-Dihydroxylabd-14-en-2-one (**3**) *from* **6**. As described for **4**→**2**, with CrO_3 (147.6 mg, 1.476 mmol), pyridine (0.233 g, 2.952 mmol), CH_2Cl_2 (15 ml), **6** (80 mg, 0.246 mmol), and pyridine (2 ml): 91 mg of colorless oily residue. Purification by CC (52 × 2 cm, silica gel (7 g), AcOEt/hexane 1:3) and crystallization from $CHCl_3$ yielded 72 mg (90%) of **3**. Colorless needles: M.p. 106–107°. R_f 0.49. $[\alpha]_D^{25} = +9.6$ ($c = 1.91$, $CHCl_3$); [12]: $[\alpha]_D^{22} = +9.2$ ($c = 1.69$, $CHCl_3$). IR: 3440, 2960, 2860, 1710, 1460, 1280, 1140, 1080, 990, 910. ^{1}H-NMR: 5.81 (*dd*, $J_{cis} = 10.7$, $J_{trans} = 17.3$, H–C(14)); 5.11 (*dd*, $J_{gem} = 1.4$, $J_{trans} = 17.3$, H–C(15) *trans* to H–C(14)); 4.90 (*dd*, $J_{gem} = 1.4$, $J_{cis} = 10.7$, H–C(15) *cis* to H–C(14)); 3.24 (br. *s*, exchangeable with D_2O, 2OH); 2.25 (*dd*, $J = 2.0, 12.3$, H–C(1)); 2.21 (*d*, $J = 13.5$, H–C(3)); 2.05 (2*d*, $J = 13.5, 12.3$, H–C(1), H–C(3)); 1.83 (*dt*, $J = 12.3, 3.2$, H–C(7)); 1.71–1.58 (*m*, H–C(12), H–C(6)); 1.52 (*m*, H–C(7)); 1.47 (*dd*, $J = 2.2, 11.5$, H–C(5)); 1.31 (*t*, $J = 3.3$, H–C(9)); 1.24 (*m*, H–C(6), H–C(11)); 1.17 (*s*, CH_3(16)); 1.08 (*s*, CH_3(17)); 0.98 (*s*, CH_3(18)); 0.77 (*s*, CH_3(19)); 0.72 (*s*, CH_3(20)). ^{13}C-NMR: *Table*. EI-MS: 304 (13, $[M - H_2O]^+$), 289 (11), 286 (6), 271 (7), 251 (6), 209 (25), 206 (22), 191 (37), 179 (33), 151 (42), 135 (59), 107 (49), 95 (49), 81 (58), 71 (100), 68 (59), 55 (71). HR-MS: 304.2403 ($C_{20}H_{32}O_2$, $[M - H_2O]^+$, calc. 304.2401).

8α,13β-Dihydroxylabd-14-en-2α-yl Acetate (**10**) *from* **6**. To a soln. of 100 mg (0.308 mmol) of **6** in 2 ml of dry pyridine and a catalytic amount of 4-(dimethylamino)pyridine at 0°, Ac_2O (5.0 ml) was added slowly and stirred at 0° for 1 h. Then, H_2O/ice was added and stirred for 30 min at r.t. The mixture extracted with AcOEt, and the org. layer washed successively with 10% HCl soln. until the aq. layer remained acidic, 10% NH_4OH soln. until the aq. layer remained basic, and H_2O, dried (Na_2SO_4), and evaporated: 111 mg of **10**. Recrystallization from AcOEt/hexane afforded 101 mg (90%) of **10**. R_f 0.65. White needles. M.p. 134–135°. $[\alpha]_D^{25} = -22.0$ ($c = 0.05$, $CHCl_3$). IR: 3400, 2980, 2880, 1735, 1460, 1390, 1250, 1030, 990. ^{1}H-NMR: 5.87 (*dd*, $J_{cis} = 10.7$, $J_{trans} = 17.3$, H–C(14)); 5.16 (*dd*, $J_{gem} = 1.3$, $J_{trans} = 17.3$, H–C(15) *trans* to H–C(14)); 4.95 (*dd*, $J_{gem} = 1.3$, $J_{cis} = 10.7$, H–C(15) *cis* to H–C(14)); 4.99 (*tt*, $J = 4.4, 11.6$, H–C(2)); 3.55 (br. *s*, exchangeable with D_2O, 2OH); 1.98 (*s*, CH_3(22)); 1.90 (*dd*, $J = 2.8, 11.7$, H–C(1)); 1.81, (*dt*, $J = 2.5, 12.0$, H–C(7)); 1.71 (*dd*, $J = 2.5, 12.2$, H–C(3)); 1.56 (*m*, H–C(12), H–C(6)); 1.45 (*m*, H–C(11)); 1.36 (*m*, H–C(7)); 1.27 (*m*, H–C(11)); 1.22 (*s*, CH_3(16)); 1.16 (*m*, H–C(3), H–C(9)); 1.11 (*s*, CH_3(17)); 0.94 (*m*, H–C(5), H–C(1)); 0.88 (*s*, CH_3(18)); 0.83 (*s*, CH_3(19), CH_3(20)). ^{13}C-NMR: *Table*. EI-MS: 348 (5, $[M - H_2O]^+$), 333 (2), 288 (6), 273 (5), 230 (3), 217 (5), 190 (20), 175 (48), 161 (16), 147 (22), 135 (24), 121 (26), 107 (28), 95 (35), 81 (39), 71 (63), 55 (32), 43 (100). HR-MS: 348.2646 ($C_{22}H_{36}O_3$, $[M - H_2O]^+$, calc. 348.2664).

8α,13β-Dihydroxylabd-14-en-3α-yl Acetate (**9**) *from* **8**. As described for **10**, from 80 mg (0.246 mmol) of **8** (1.5 h): 89 mg of **9**. Purification by CC (52 × 2 cm, silica gel (5 g)) and recrystallization from AcOEt/hexane yielded 82 mg (91%) of **9**. R_f 0.55. White crystals. M.p. 143–144°. $[\alpha]_D^{25} = -33.0$ ($c = 0.1$, $CHCl_3$). IR: 3340, 2940, 2880, 1735, 1460, 1415, 1380, 1255, 1140, 1050, 990. ^{1}H-NMR: 5.93 (*dd*, $J_{cis} = 10.7$, $J_{trans} = 17.4$, H–C(14)); 5.21 (*dd*, $J_{gem} = 1.3$, $J_{trans} = 17.4$, H–C(15) *trans* to H–C(14)); 5.02 (*dd*, $J_{gem} = 1.3$, $J_{cis} = 10.7$, H–C(15) *cis* to H–C(14)); 4.62 (*dd*, $J = 2.7, 2.7$, H–C(3)); 2.20 (br. *s*, exchangeable with D_2O, 2OH); 2.07 (*s*, CH_3(22)); 1.85 (*dt*, $J = 2.8, 11.9$, H–C(7)); 1.65 (*m*, H–C(12), H–C(2)); 1.53 (*m*, H–C(6)); 1.40 (*m*, H–C(7)); 1.36 (*m*, H–C(5), H–C(1)); 1.27 (*s*, CH_3(16)); 1.22 (*t*, $J = 3.5$, H–C(9)); 1.16 (*s*, CH_3(17)); 0.85 (*s*, CH_3(19)); 0.84 (*s*, CH_3(18)); 0.80 (*s*, CH_3(20)). ^{13}C-NMR: *Table*. EI-MS: 348 (2, $[M - H_2O]^+$), 333 (4), 330 (2), 288 (4), 273 (6), 255 (6), 235 (5), 219 (8), 203 (9), 190 (36), 175 (74), 161 (31), 147 (42), 135 (67), 121 (52), 107 (54), 95 (51), 81 (60), 71 (100). HR-MS: 348.2649 ($C_{22}H_{36}O_3$, $[M - H_2O]^+$, calc. 348.2664).

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