MeOH gradient and 500 ml fractions were collected. The toxic fractions (25 and 26, 712 mg total) eluted with $CHCl_3$–Me_2CO (4:1) were combined and further separated by gel filtration on a 110 × 2 cm column of Sephadex LH-20 with $CHCl_3$–MeOH (1:1). The solid (52 mg) eluted after the toxic oil was dissolved in CH_2Cl_2–hexane and 13 mg of (+)-α(*S*)-butyramido-γ-butyrolactone crystallized from the cooled soln as fine white needles, mp 120–121°; $[\alpha]_D^{25}$ = +18.9° ($CHCl_3$, *c* 0.74); PMR (1:1 $CDCl_3/C_6D_6$) δ 0.80 (*t*, *J* = 7 Hz, Me), 1.52 (*m*, *J* = 7 Hz, CH_2=Me), 1.92 (*t*, *J* = 7 Hz, CH_2=C=O), 1.92 (*m*, C-3 H), 2.13 (*dddd*, *J* = 2, 6, 9 and 12 Hz, C-3 H), 3.56 (*ddd*, *J* = 6, 9 and 12 Hz, C-4 H), 3.82 (*ddd*, *J* = 2, 9 and 9 Hz, C-4 H), 4.26 (*ddd*, *J* = 6, 9 and 12 Hz, C-2 H), 6.1 (*bd*, *J* = 6 Hz, NH); C^{13} NMR ($CDCl_3$) δ 13.6 (Me), 18.8 (CH_2), 30.3 (CH_2), 37.9 (CH_2), 49.0 (CH_2), 66.0 (CH), 173.4 (C=O), 175.4 (C=O); IR ($CHCl_3$) ν_{max} 3425, 2920, 2870, 1780, 1676 cm^{-1}; MS *m/e* (rel. intensity) 171 (11), 153 (7), 143 (73), 71 (88), 43 (100), 28 (95); high resolution mass measurement 171.088690 (calcd for $C_8H_{13}NO_3$ 171.089547).

Hydrolysis The natural (+)-α(*S*)-butyramido-γ-butyrolactone (10 mg) in 2N HCl was refluxed for several hr. The odor of *n*-butyric acid was detected. Evapn. of the solvent gave α-amino-γ-butyrolactone HCl which exhibited a PMR spectrum in D_2O that was identical to that of a commercial sample of α-amino-γ-butyrolactone hydrobromide. No optical rotation, however, could be detected.

Synthesis. Racemic α-butyramido-γ-butyrolactone prepared from α-amino-γ-butyrolactone hydrobromide following the procedure of ref. [3] melted at 83°. (+)-α(*S*)-Butyramido-γ-butyrolactone prepared from (−)-α(*S*)-amino-γ-butyrolactone hydrobromide [4] had mp 120–121° and $[\alpha]_D^{25}$ +21.1° ($CHCl_3$, *c* 0.18).

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SPHINGOSINE DERIVATIVES FROM RED ALGAE OF THE CERAMIALES

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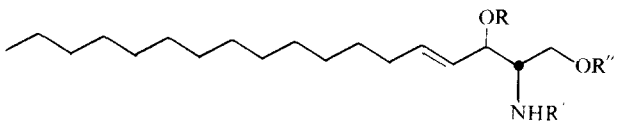
Key Word Index—*Laurencia nidifica*; *Amansia glomerata*, Rhodomelaceae; dihydrosphingosine *N,O*-diacetate; *N*-acylsphingosines.

Abstract—(+)-2(*S*)-*N*-Acetamido-3(*R*)-acetoxyoctadecan-1-ol, a diacetate of dihydrosphingosine, and fatty acid amides of (−)-2(*S*)-amino-3(*R*)-hydroxyoctadec-4(*E*)-en-1-ol (sphingosine) have been isolated from extracts of Hawaiian *Laurencia nidifica* and *Amansia glomerata*, respectively. Although well known as constituents of nerve tissue hydrolyzates throughout the animal kingdom, these compounds have not been previously found in plants.

INTRODUCTION

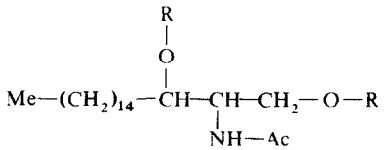
Sphingosine (**1a**) has been known since 1882 [1] to be a component of brain tissue hydrolyzate but its structure was not established until 65 years later [2]. This unsaturated amino dialcohol has been found in the hydrolyzates of rat, beef and human brain tissues In 1941, Lesuk and Anderson [3] isolated dihydrosphingosine (**2a**) from hydrolyzed larvae (*Cysticercus fasciolaris*) of *Taenia taeniaformis*, the common tapeworm of cats. Dihydrospingosine compounds were later [4] shown to be present in beef spinal cords.



1a R = R′ = R″ = H
1b R = R″ = H, R′ = $COCH_2(CH_2)_nMe$ where *n* = 12, 14, 16, 20, 21, 22

RESULTS AND DISCUSSION

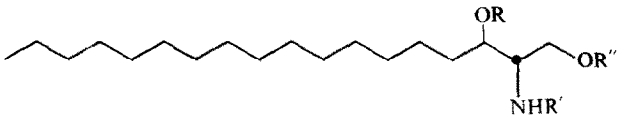
While examining the more polar extracts of *Laurencia nidifica*, we isolated (+)-2(*S*)-*N*-acetamido-3(*R*)-acetoxyoctadecan-1-ol (**2b**), a diacetate of dihydrosphingosine. The IR spectrum provided evidence for alcohol, ester, and amide functionalities and the PMR spectrum indicated that these 3 groups were at one end of an aliphatic chain. Spin–spin decoupling experiments suggested partial structure *A*. The PMR spectrum of the triacetate derivative indicated a paramagnetic shift of a two proton multiplet (3.58 in **2b** → *ca* 4.2 in **2c**), fixing the position of the OH group as shown in **2b**.



A

The MS of **2b** showed fragment ions at *m/e* 354 for loss of CH_2OH from the M^+ and *m/e* 102 for $AcNH^+{=}CH{-}CH_2OH$. The physical constants of the corresponding triacetate (**2c**) were comparable with previously published values [4].

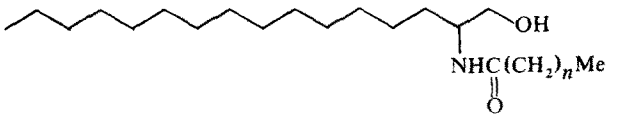
A mixture of related compounds was found in the polar extracts of *Amansia glomerata*. PMR and MS data indicated that these compounds were amides. Hydrolysis led to sphinogosine and a mixture of unbranched, saturated fatty acids. The sphingosine was converted to the triacetate and identified by direct comparison with an authentic sample. MS analysis



2a R = R′ = R″ = H
2b R″ = H, R = R′ = Ac
2c R = R′ = R″ = Ac

of the fatty acid showed that it was composed of predominately *n*-C_{23}, C_{24} and C_{25} acids with smaller amounts of C_{14}, C_{16} and C_{18} acids.

To our knowledge, this is the first case in which sphingosine derivatives have been found in plants. It is interesting to note the similarity of these compounds to caulerpicin (**3**), a mixture of *N*-acyl-2-aminohexadecan-1-ols from the green alga *Caulerpa racemosa* [5].



n = 23, 24, 15

EXPERIMENTAL

PMR and C^{13}NMR spectra were determined on a 100 MHz spectrometer; chemical shifts are reported in δ units (ppm) relative to TMS (δ = 0) as an int. stand. in $CDCl_3$. MS were obtained at 70 eV. Elemental analysis were performed by Chemical Analytical Services, University of California, Berkeley.

Isolation of **2b**. *Laurencia nidifica* (575 g dry wt), collected near Port Allen, Kauai, was extracted successively with Me_2CO and hexane. The combined extracts were concd and partitioned between hexane and H_2O. The hexane soluble material (17.3 g) was chromatographed on a column of Si gel (62 × 5 cm). Eluting successively with solvents of gradually increasing polarity (hexane, THF, Me_2CO, MeOH) 12 fractions were collected. Fractions 9 and 10 (587 mg), eluted with Me_2CO and Me_2CO–MeOH (1:1) were combined and rechromatographed on neutral Al_2O_3. Elution with CH_2Cl_2–MeOH (97.3) removed 56 mg of a tan solid which was recrystallized from CH_3CN–H_2O to give 45 mg of (+)-2(*S*)-*N*-acetamido-3(*R*)-acetoxyoctadecan-1-ol (**2b**) as white crystals, mp 91.5–92.5°; $[\alpha]_D$ +10.8 (CH_2Cl_2, *c* 1.25); PMR δ 0.85 (*bt*, C-Me), 1.23 (*br s*, $-(CH_2)_n-$), 1.6 (*m*, $-CH_2-C(=O)-$), 1.99 (*s*, $-NCOMe$), 2.09 (*s*, $-OAc$), 3.04 (*br s*, $-OH$), 3.58 (*bd*, $-CH_2O$), 4.02 (*m*, $-CH-N$), 4.86 (*bq*, $-CH-O$), 6.31 (*bd*, $-NH-$); C^{13}NMR δ 172.17 (C=O), 170.32 (C=O), 73.97 (CH), 61.65 (CH), 53.46 (CH_2), 31.97 (CH_2), 31.35 (CH_2), 29.77 (10 CH_2), 25.71 (CH_2), 23.43 (CH_2), 22.81 (Me), 21.05 (Me), 14.18 (Me); IR (KBr) 3380, 3280, 3060, 1729, 1642, 1540, 1235 cm^{-1}; MS *m/e* 386 (M + 1) 354, 324, 295, 294, 270, 157, 145, 102; high resolution MS *m/e* 386.324979 (calcd for $C_{22}H_{44}NO_4$, 386.3271). Analysis. Found C, 68.35; H, 11.12; N, 3.56. Calcd. for $C_{22}H_{43}NO_4$: C, 68.53; H, 11.24; N, 3.63.

Acetylation of **2b**. Acetylation of 19 mg of **2b** with Ac_2O and Py and recrystallization of the product from MeCN gave 11.2 mg of dihydrosphingosine triacetate (**2c**) as white crystals, mp 100.5–101°, $[\alpha]_D$ +15.7 ($CHCl_3$, *c* 0.86) [lit. [4] mp 102–103°, $[\alpha]_D$ +18.1 ($CHCl_3$, *c* 1.0)]; PMR δ 0.86 (*bt*, 3H), 1.23 (*br s*, 26 H), 1.6 (*m*, 2H), 1.98 (*s*, 3H), 2.04 (*s*, 3H), 2.06 (*s*, 3H), 3.96–4.52 (*m*, 3H), 4.87 (*m*, 1H), 5.88 (*b*, 1H).

Isolation of **1b**. *Amansia glomerata* was collected at Black Point, Oahu and dried at 40°. The dried seaweed (1.35 kg) was extracted successively with petrol and Me_2CO. The Me_2CO extract (4.5 g) was chromatographed on neutral Al_2O_3 with a solvent gradient of hexane, $CHCl_3$ and MeOH. 9 fractions were collected. Fractions 4 and 5 eluted with $CHCl_3$–MeOH (99:1), were combined (115 mg). Filtration through Sephadex LH-20 ($CHCl_3$–MeOH, 3:2) and recrystallization from MeCN–EtOAc gave a mixture of *N*-acylsphingosines (**1b**) as an amorphous white solid, mp 78–81° $[\alpha]_D$ −5.0° ($CHCl_3$, *c* 1.2).

Hydrolysis of **1b**. The mixture of *N*-acylspingosines (**1b**, 63 mg) was hydrolyzed with 30 ml 1.2 M H_2SO_4 in 85% MeOH at reflux for 4 hr [6]. The fatty acid Me esters (43 mg) were extracted from the hydrolyzate with heptane and the aq. phase was then basified with 2M NaOH and extracted with heptane–CH_2Cl_2 (1:1) to obtain 12 mg of crude sphingosine. The fatty acid esters were separated into two fractions by gel filtration on Sephadex LH-20. One fraction (34 mg) contained *n*-C_{23} (MS *m/e* 354, 323), *n*-C_{24} (*m/e* 368, 337), and *n*-C_{25} (*m/e* 382, 351) acid Me esters in a ratio of *ca* 4.1.2; the other fraction (9 mg) contained tetradecanoic (*m/e* 242, 211), (*m/e* 270, 239) and octadecanoic (*m/e* 298, 267) acid Me esters in a ratio of *ca* 5:6:1. The crude sphingosine was converted to the triacetate (Ac_2O, NaOAc) The product (11 mg) $[\alpha]_D$ −8.4 ($CHCl_3$, *c* 0.83) [lit. [4] $[\alpha]_D$ −11.7 ($CHCl_3$, *c* 1.0)] gave a PMR spectrum which was identical to that of authentic sphingosine triacetate. δ 0.84 (*bt*, 3H), 1.24 (*br s*, 22H), 1.4 (*m*, 2H), 1.96 (*s*, 3H), 2.04 (*s*, 3H), 2.05 (*s*, 3H), 3.9–4.6 (*m*, 3H), 5.1–5.95 (*m*, 4H).

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