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PINNATANINE AND OXYPINNATANINE, NOVEL AMINO ACID AMIDES FROM *STAPHYLEA PINNATA* L.

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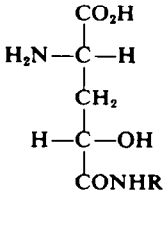
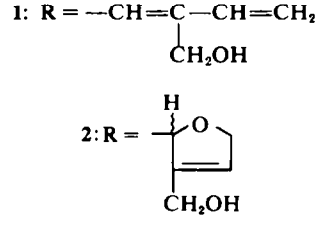
Abstract—Two novel amino acid amides, pinnatanine and oxypinnatanine, have been isolated from *Staphylea pinnata* L. Their respective structures have been established as N^5-(2-hydroxymethylbutadienyl)-L-*allo*-γ-hydroxyglutamine and N^5-(3-hydroxymethyl-2,5-dihydro-2-furyl)-L-*allo*-γ-hydroxyglutamine by spectral methods and chemical degradation.

Seed from the shrub *Staphylea pinnata* L. (European bladdernut, family Staphyleaceae) was reported by VanEtten *et al.*[1] to exhibit a most unusual amino acid composition when compared with seed from 200 other angiospermous plant species. In addition to unusually high amounts of isoleucine and low amounts of several common amino acids, two unidentified component acids were prominent in the hydrolysate of *S. pinnata* seed meal. Detailed investigation of unhydrolyzed seed meal has shown it to be a rich source of two novel derivatives of γ-hydroxyglutamine. The first compound isolated, designated as pinnatanine and characterized as N^5-(2-hydroxymethylbutadienyl)-L-*allo*-γ-hydroxyglutamine (1), was the subject of a recent communication.[2] We now report studies on a second amide, oxypinnatanine, which has been identified as N^5-(3-hydroxymethyl-2,5-dihydro-2-furyl)-L-*allo*-γ-hydroxyglutamine (2) as well as experimental details of the isolation and characterization of 1.





Extraction of *S. pinnata* defatted seed meal with aqueous EtOH, followed by column chromatography on silica gel using aqueous *iso*-PrOH, gave the two crystalline amides, which accounted for 20% of the partially refined extract applied to the column. Isolated pinnatanine and oxypinnatanine represent 3·5% and 2·9%, respectively, of the defatted meal.

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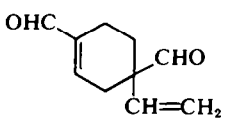
Upon acid hydrolysis, both pinnatanine (1), $C_{10}H_{16}N_2O_5$, and oxypinnatanine (2), $C_{10}H_{16}N_2O_6$, afforded the $C_5H_9NO_5$ amino acid, L-*allo*-γ-hydroxyglutamic acid [i.e., 2(*S*), 4(*S*)]. The acid hydrolysate from each compound also contained a CH_2Cl_2 soluble product which accounted for the remaining five C atoms.

For pinnatanine, a five-C fragment was not isolated as such, but rather a $C_{10}H_{12}O_2$ unsaturated dialdehyde was obtained. Identification of this dialdehyde as 4-vinyl-1-cyclohexene-1,4-dicarboxaldehyde (3) was based on its IR, NMR and mass spectral (MS) properties and by direct comparison with an authentic sample prepared by synthesis.[3] Compound 3 presumably was formed via Diels-Alder dimerization of 2-methylene-3-butenal, the hydrolysis product expected on the basis of interpretation of NMR, UV and compositional data on 1.[2]

Acetylation of pinnatanine gave diacetyl lactone 4 whose NMR spectrum exhibited NH-proton doublets at δ 7·20 and 8·87, which disappeared upon addition of 1N NaOD. Spin decoupling experiments with 4 showed that the δ 7·20 signal was coupled to the α-proton of the amino acid lactone at δ 4·51 and that the δ 8·87 proton was coupled to the lowfield vinyl proton doublet at δ 6·92. These results demonstrated that the hydroxymethylbutadienyl moiety is attached to the amide nitrogen rather than to the nitrogen or oxygen of the γ-hydroxyglutamyl portion. Evidence that pinnatanine is an α-amino-γ-hydroxy acid (1) and not an α-hydroxy-γ-amino acid was provided by measuring the apparent dissociation constant of the amino group and a downfield shift in the NMR signal for the α-proton at pH 1.[2]

The NMR spectrum of oxypinnatanine (D_2O) exhibited two one-proton multiplets at δ 3·92 and 4·33 (q, $J = 5·5$ and 7·5 Hz) coupled to a two-proton multiplet at δ 2·26, a pattern essentially identical to that observed with 1 due to the γ-hydroxy-



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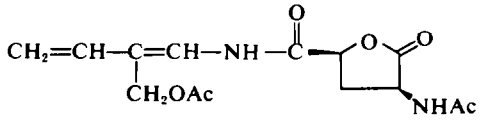

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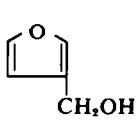
glutamyl moiety. Six additional non-D_2O exchangeable protons appeared as a two-proton singlet at δ 4·19, a two-proton multiplet at δ 4·65 and two one-proton multiplets at δ 6·17 and 6·37. When the spectrum was measured at 50°, the HOD signal shifted 0·2 ppm upfield to δ 4·52 and allowed the δ 4·65 signal to be observed. Irradiation at δ 4·65 collapsed the δ 6·17 multiplet to a doublet and sharpened the δ 6·37 signal. Upon simultaneous irradiation at δ 4·19 and 4·65, both one-proton multiplets collapsed to doublets (J = 1·5 Hz).

The UV spectrum of oxypinnatanine showed only end absorption, which contrasted sharply with the strong UV maximum of 1 at 262 nm. These spectral data suggested a tentative structure for oxypinnatanine quite similar to pinnatanine in which the additional oxygen atom might be accommodated by a dihydrofuran ring as depicted by structure 2. With such a structure, the NMR signal at δ 4·65 could then be ascribed to the dihydrofuran methylene, and the signal at δ 4·19 would be indicative of an allylic alcohol methylene.

The CH_2Cl_2 soluble portion of the acid hydrolysate of oxypinnatanine consisted of an oil which was not a dihydrofuran. The NMR spectrum ($CDCl_3$) exhibited signals at δ 7·33 (two protons) and 6·37 (one proton) attributed to the α- and β-protons, respectively, of a 3-substituted furan. In addition, a two-proton singlet at δ 4·45, plus OH absorption in the IR at 3610 cm^{-1}, suggested that the hydrolysis product was 3-furylcarbinol (5). Direct comparison (TLC, IR, NMR and MS) of this product with an authentic sample obtained by LAH reduction of ethyl furan-3-carboxylate[4] showed them to be identical. Furan 5 presumably resulted from a facile acid-promoted aromatization of an intermediate 2-amino- or 2-hydroxy-dihydrofuran.



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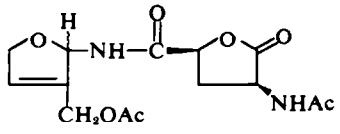

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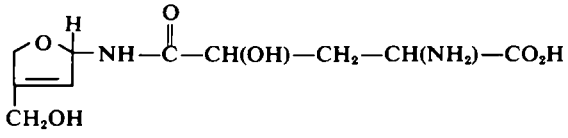
Acetylation of oxypinnatanine gave a crystalline diacetyl lactone 6 ($C_{14}H_{18}N_2O_7$), which had IR bands at 1795 (γ-lactone) and 1740 cm^{-1} (ester C=O). Its NMR spectrum ($CDCl_3$) exhibited two acetyl methyl singlets at δ 1·99 and 2·05 and two NH-proton doublets at δ 6·85 (J = 7 Hz) and 7·36 (J = 9·5 Hz). Upon irradiation at δ 7·36, a broad one-proton multiplet at δ 6·42 sharpened and could be assigned to the C-1 proton of the dihydrofuran ring. An unresolved six-proton complex appeared at δ 4·5–4·9, which included the dihydrofuran and acetoxy methylenes and two lactone methine protons. Irradiation at δ 4·68 collapsed the NH-proton doublet at δ 6·85 to a singlet; the δ 6·42 multiplet to a quartet (J = 1·5 and 9·5 Hz); and a δ 6·11 multiplet, assigned to the dihydrofuran vinyl proton, to a doublet (J = 1·5 Hz). Signals observed at δ 6·42 and 6·11 with 6 correspond to the δ 6·37 and 6·17 resonances exhibited by oxypinnatanine. These results show that the nonamino acid portion of oxypinnatanine, as in pinnatanine, is attached to the amide nitrogen of γ-hydroxyglutamine.

Isomeric structure 7, which could also yield 5 upon hydrolysis, was eliminated from consideration by degrading oxypinnatanine to the monodeuterated 2-methyl-1*d*-1,4-butanediol unobtainable from 7. This result established 2 as the correct formula for oxypinnatanine, which has the same carbon skeleton as 1.

The precursor (8) required for degradative studies was obtained in 75% yield by hydrogenolysis of the allylic acetate 6. The product was isolated by fractional crystallization and had no ester carbonyl absorption in the IR. Its NMR spectrum (CD_3OD) contained a methyl doublet at δ 1·07 (J = 6 Hz) and one acetyl methyl singlet at δ 1·98. The C-1 proton of the tetrahydrofuran appeared as a doublet at δ 5·16 (J = 5·5 Hz).

A model compound, 2-methyl-1,4-butanediol diacetate (9), was prepared by $NaBH_4$ reduction of α-methyl-γ-butyrolactone, followed by acetylation of the resulting diol. The NMR spectrum of 9 showed the C-1 methylene as a doublet at δ 3·92 and the C-4 methylene as a triplet at δ 4·11. Although the molecular ion at *m/e* 188 was absent in the MS of 9, an M+1 ion at *m/e* 189 was



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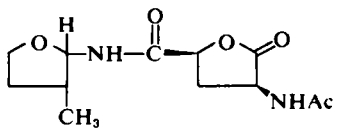

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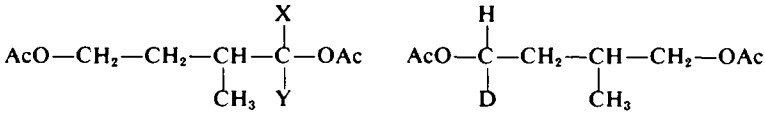

9: X = Y = H

10: X = Y = D

11: X = H, Y = D

12

observed because sample pressure was excessive.[5] The base peak appeared at *m/e* 68 (M − 2 HOAc). Reduction of the lactone with $NaBD_4$ led to the dideuterated diacetate **10**, which gave an NMR spectrum that lacked a δ 3·92 signal yet exhibited the expected two-proton triplet at δ 4·11. The MS showed an M + 1 ion at *m/e* 191 and a base peak at *m/e* 70.

Hydrolysis of **8** followed by $NaBD_4$ reduction and acetylation should yield **11**. Similar degradation of the corresponding hydrogenolysis product from **7** would, however, give **12**, which could be distinguished from **11** by comparison of integrated NMR signals at δ 3·92 and 4·11. Application of this degradation scheme to the hydrogenolysis product from acetylated oxypinnatanine afforded a diacetate whose MS gave an M + 1 ion at *m/e* 190 and a base peak at *m/e* 69 (M − 2 HOAc) indicating incorporation of one deuterium. Since the NMR spectrum showed a one-proton doublet, broadened by H-C-D coupling, at δ 3·91 and a two-proton triplet, at δ 4·11, structure **11** for the degradation product was established.

The occurrence of these novel derivatives of γ-hydroxyglutamine is not restricted to *S. pinnata*. Both **1** and **2** are also present in seed of *Hemerocallis fulva* L. (common orange day lily, family Liliaceae) as shown by paper chromatography and NMR. Oxypinnatanine is likely the same as a $C_{10}H_{16}N_2O_6$ amino acid reported several years ago in *Phlox decussata* (family Polemoniaceae).[6] This compound also gave γ-hydroxyglutamic acid and ammonia upon hydrolysis; however, the remainder of the molecule was not identified.

EXPERIMENTAL

All m.ps were determined on a Fisher-Johns* block and are uncorrected. Optical rotations were taken on a Bendix 1169 polarimeter. The following spectrometers were used: UV, Beckman DK-2A; IR, Perkin-Elmer 337; NMR, Varian HA-100 with TMS or TSP [sodium 2,2,3,3,-tetradeutero-3-(trimethylsilyl)-propionate] as internal standards and chemical shifts reported in δ (ppm) units; MS, Du Pont (CEC) 21-492-1. GLC-MS analysis was done as previously described[7] on a 2 ft × ⅛ in. 5% Apiezon L column programed from 50 to 230° at 5°/min. TLC analyses were carried out on Silica Gel G-coated plates.

Extraction and preliminary fractionation. Light petroleum defatted seed meal (15 g) from *Staphylea pinnata* was extracted twice at room temp for 1 hr with EtOH-H_2O (7:3) and once with abs EtOH. The combined extract was concentrated to near dryness, taken up in warm H_2O (50 ml), and extracted with Et_2O (5 × 125 ml). The concentrated aqueous phase (4·98 g) was chromatographed on silica gel (270 g, 70–325 mesh) and the following 50-ml fractions were collected: 1–34, iso-PrOH—H_2O (9:1); 35–43, (8·5:1·5); 44–70 (4:1). TLC analysis using iso-PrOH—EtOAc—H_2O—HOAc (40:38:20:2) followed by spraying with ninhydrin [0·5 g in HOAc—*n*-BuOH—acetone (3:50:50)] and heating at 110° revealed two major red zones.

Pinnatanine (**1**). Silica gel column fractions 10–26 were combined, partially concentrated, and refrigerated to give colorless needles of **1** (526 mg): m.p. dec. starts 175°; $[\alpha]_D^{27}$ + 3·2° (*c*, 0·5, H_2O), TLC R_f 0·4; UV (H_2O) max 262 nm (ε 24,200); IR (KBr) 3385 and 3335 (OH and NH), 1655 (amide I C=O), 1625 (—CO_2^-), 1600 (C=C), 1500 cm^{-1} (amide II C=O); NMR (D_2O) δ 2·28 (2H, m, O—CH—$\underline{CH_2}$—CH—N), 3·94 (1H, q, *J* = 5 and 6 Hz, CH_2—$\underline{CH}$—N), 4·29 (2H, s, C=C—CH_2—O), 4·45 (1H, q, *J* = 5 and 8 Hz, CH_2—$\underline{CH}$—O), 5·31 and 5·45 (1H each, two d, *J* = 11 and 17 Hz, $\underline{CH_2}$=CH), 6·65 (1H, q, *J* = 11 and 17 Hz, CH =$\underline{CH}$), 6·74 (1H, s, C=CH—N). (Found: C, 49·12; H, 6·66; N, 11·30. $C_{10}H_{16}N_2O_5$ requires: C, 49·18; H, 6·55; N, 11·51%).

Acid hydrolysis of pinnatanine (**1**). A soln of **1** (250 mg) in 2N H_2SO_4 (10 ml) was heated under reflux for 3 hr, cooled, and extracted with CH_2Cl_2. The aqueous phase was decolorized with charcoal, filtered through Celite, and made alkaline to pH 9 with 2N NaOH. The soln was allowed to stand for ½ hr, acidified to pH 3·5 with 2N HCl, and concentrated. An aqueous soln of the concentrate was applied to a column of Bio-Rad AG 50W-X8 cation exchange resin (acid form, 50 g) which was eluted with H_2O (375 ml) and 2N NH_3. Ninhydrin positive fractions eluted by NH_3 were combined, concentrated, and applied to a column of AG 1-X4 anion exchange resin (acetate form, 5 g). The column was eluted with H_2O (120 ml) and 0·5 N HOAc. Ninhydrin positive fractions eluted by HOAc were combined, partially concentrated, and refrigerated. The resulting crystals were recrystallized from EtOH-H_2O to give L-*allo*-γ-hydroxyglutamic acid (65 mg): m.p. 182–185° dec.; $[\alpha]_D^{27}$ − 13·2° (*c*, 0·5, H_2O) (lit.[8] $[\alpha]_D^{26}$ − 13·6°); IR (KBr) 1715 (acid C=O), 1630 cm^{-1} (-CO_2^-), superimposable on that of an authentic sample; NMR (D_2O) δ 2·31 (2H, m, O—CH—$\underline{CH_2}$—CH—N), 3·92 (1H, m, CH_2—$\underline{CH}$—N), 4·32 (1H, m, CH_2—$\underline{CH}$—O). (Found: C, 36·71; H, 5·68; N, 8·41. $C_5H_9NO_5$ requires: C, 36·81; H, 5·56; N, 8·59%).

*The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

The CH_2Cl_2 soluble portion of the hydrolysate was dried (Na_2SO_4), concentrated, and chromatographed on silica gel (3 g). The major component (50 mg) was eluted with benzene-acetone (99:1) to give **3** as a yellow oil: TLC R_f 0·4 [benzene-acetone (95:5), sprayed with 3% ceric sulfate in 3N H_2SO_4 and heated at 120°]; UV (EtOH) max 230 nm (ϵ 14,500); IR ($CHCl_3$) 2815 and 2715 (aldehyde CH), 1725 (aldehyde C=O), 1680 (α,β-unsaturated aldehyde C=O), 1645 (C=C), 994 and 917 cm^{-1} (CH_2=CH); NMR ($CDCl_3$) δ 1·62–2·96 (6 H, m, three CH_2), 5·14 and 5·32 (1H each, two q, J = 1 and 17 Hz and J = 1 and 11 Hz, $\underline{CH_2}$=CH), 5·74 (1 H, q, J = 11 and 17 Hz, CH_2=$\underline{CH}$), 6·80 (1H, m, CH_2—$\underline{CH}$=C), 9·36 (1H, s, CHO), 9·40 (1H, s, CHO); MS *m/e* 164 (M^+, $C_{10}H_{12}O_2$); Dioxime, m.p. 133·5–137°.

4-Vinyl-1-cyclohexene-1,4-dicarboxaldehyde (**3**). Compound **3** was prepared by NaOAc-catalyzed condensation of crotonaldehyde with formaldehyde as described by Pummerer *et al.*[3] The benzene extract from the reaction mixture was purified by filtration through silica gel in $CHCl_3$ followed by distillation through a spinning band column to give **3**: b.p. 80° (0·25 mm); Dioxime, m.p. 135·5–137°.

Acetylation of pinnatanine (**1**). A soln of **1** (74 mg) in Ac_2O-pyridine (3 ml each) was kept at room temp for 2 days. The mixture was concentrated to dryness, taken up in $CHCl_3$, and chromatographed on silica gel (10 g). Elution with $CHCl_3$-acetone (4:1) afforded **4** as a yellow oil (39 mg): TLC R_f 0·4 [$CHCl_3$-acetone (1:1), I_2 vapor]; UV (EtOH) max 266 nm; IR ($CHCl_3$) 3460 and 3430 (amide NH), 1795 (γ-lactone), 1735 (ester C=O), 1690 (amide I C=O), 1660 (amide I C=O), 1490 cm^{-1} (amide II C=O); NMR ($CDCl_3$) δ 2·01 and 2·04 (3H each, two s, Ac), 2·30 and 3·00 (1H each, two m, O—CH—$\underline{CH_2}$—CH—N), 4·51 (1H, m, CH_2—$\underline{CH}$—NHAc), 4·73 (2H, s, C=C—CH_2—OAc), 4·93 (1 H, q, J = 7·5 and 9·0 Hz, CH_2—$\underline{CH}$—O—CO), 5·22 and 5·33 (1 H each, two d, J = 11 and 17 Hz, $\underline{CH_2}$=CH), 6·66 (1H, q, J = 11 and 17 Hz, CH_2=$\underline{CH}$), 6·92 (1 H, d, J = 11 Hz, C=$\underline{CH}$—NHAc), 7·20 (1 H, d, J = 7 Hz, CH—$\underline{NH}$Ac), 8·87 (1 H, d, J = 11 Hz, C=CH—$\underline{NH}$Ac).

Oxypinnatanine (**2**). Silica gel column fractions 36-56 (see Extraction and preliminary fractionation) were combined, concentrated, and redissolved in a small volume of H_2O. The soln was chromatographed on silica gel (130 g) using iso-PrOH—H_2O (9:1, 1 liter and 8·5:1·5, 900 ml). The 85% iso-PrOH eluate was decolorized with charcoal, filtered through Celite, and concentrated. Crystallization from EtOH-H_2O gave **2** (441 mg): m.p. 182–185° dec; $[\alpha]^{24}$ + 5·5° (*c*, 0·8, H_2O); TLC R_f 0·2; IR (KBr) 3440 and 3260 (OH and NH), 1665 (amide I C=O), 1635 ($-CO_2^-$), 1515 cm^{-1} (amide II C=O). (Found: C, 46·38; H, 6·53; N, 10·70. $C_{10}H_{16}N_2O_6$ requires: C, 46·15; H, 6·20; N, 10·77%.)

Acid hydrolysis of oxypinnatanine (**2**). A soln of **2** (260 mg) in 2N H_2SO_4 (10 ml) was heated on a steam bath for 10 min, allowed to stand at room temp for 3 hr, and extracted with CH_2Cl_2. The amino acid component in the aqueous phase was isolated as described for the acid hydrolysis of **1** to give L-*allo*-γ-hydroxyglutamic acid (54 mg): m.p. 179–183° dec; $[\alpha]_D^{24}$ − 15° (*c*, 0·5, H_2O); IR superimposable on that of an authentic sample.

The hydrolysis of **2** (78 mg) was repeated, except heat was omitted. Concentration of the dried (Na_2SO_4) CH_2Cl_2 extract gave **5** as an oil: TLC R_f 0·3 [$CHCl_3$-acetone (95:5), I_2 vapor]; IR (CH_2Cl_2) 3610 (OH), 1595w, 1500 (furan), 1022, 965, 872 cm^{-1} (furan); MS *m/e* (%) M^+ 98(100), 97(41), 81(25), 70(20), 69(36). A sample of **5** prepared by LAH reduction of ethyl furan-3-carboxylate[4] had identical spectral properties (IR, NMR, and MS).

Acetylation of oxypinnatanine (**2**). A soln of **2** (156 mg) in Ac_2O-pyridine (5 ml each) was kept at room temp for 2 days. The mixture was concentrated to dryness, taken up in $CHCl_3$ and chromatographed on silica gel (9 g). The product eluted with $CHCl_3$-acetone (7:3) was crystallized from acetone-hexane to give **6** (99 mg): m.p. 167–168°; IR ($CHCl_3$) 3470 and 3420 (amide NH); 1795 (γ-lactone), 1740 (ester C=O), 1690 (amide I C=O), 1490 cm^{-1} (amide II C=O). (Found: C, 51·57; H, 5·60; N, 8·44. $C_{14}H_{18}N_2O_7$ requires: C, 51·53; H, 5·56; N, 8·59%).

Hydrogenolysis of acetylated oxypinnatanine (**6**). A soln of **6** (42 mg) in EtOH (8 ml) was hydrogenated with 10% Pd/C catalyst (42 mg) at atmos pressure. Absorption of H_2 ceased within 15 min, and 1·75 mol equivs were consumed. The catalyst and solvent were removed, and the product was recrystallized several times from acetone-hexane to give **8**: m.p. 211–214°; IR ($CHCl_3$) 3465 and 3420 (amide NH), 1795 (γ-lactone), 1685 (amide I C=O), 1495 cm^{-1} (amide II C=O).

2-Methyl-1,4-butanediol diacetate (**9**). A soln of $NaBH_4$ (380 mg) in H_2O (5 ml) was added dropwise to α-methyl-γ-butyrolactone (1·0 g) in H_2O (10 ml). The mixture was stirred at room temp for 1 hr, cooled, acidified to pH 1 with 2N H_2SO_4, and washed with $CHCl_3$. The aqueous phase was saturated with NaCl and extracted with EtOAc. Chromatography of the dried (Na_2SO_4) concentrated extract on silica gel (25 g) [$CHCl_3$-acetone (7:3) eluent] gave the diol as a colorless oil (292 mg): IR ($CHCl_3$) 3605 and 3415 cm^{-1} (OH). A soln of the diol (42 mg) in Ac_2O-pyridine (2 ml each) was kept at room temp overnight. The mixture was worked up in the usual manner and chromatographed on silica gel (3 g). Elution with $CHCl_3$ gave **9** as a colorless oil (39 mg): IR ($CHCl_3$) 1730 cm^{-1} (ester C=O); NMR ($CDCl_3$) δ 0·96 (3H, d, J = 6·5 Hz, $\underline{CH_3}$—CH), *ca* 1·7 [3 H, m, CH_2—$\underline{CH_2}$—$\underline{CH}$—(CH_3)—CH_2], 2·01 and 2·02 (3 H each, two s, Ac), 3·92 (2H, d, J = 6 Hz, AcO—$\underline{CH_2}$—CH), 4·11 (2H, t, J = 6·5 Hz, AcO—$\underline{CH_2}$—CH_2); MS *m/e* (%) 189 (M^+ + 1; 1), 115(10), 100(19), 98(12), 86(15), 85(42), 73(16), 72(15), 69(18), 68(100), 67(10), 61(14), 56(51), 55(21).

2-Methyl-1,1-d_2-1,4-butanediol diacetate (**10**). Reduction of the lactone with $NaBD_4$ was carried out as described above except the mixture was stirred for 3 hr. Chromatography of the EtOAc extract afforded the deuterated diol: IR ($CHCl_3$) 3620 and 3415 (OH), 2185 and 2085 cm^{-1} (C—D). Acetylation of the diol (100 mg) gave **10** (98 mg); IR ($CHCl_3$) 1730 cm^{-1}; MS *m/e* (%) 191 (M^+ + 1; 1), 115(7), 103(8), 101(12), 98(7), 88(5), 87(17), 86(23), 75(9), 73(5), 72(12), 71(12), 70(100), 69(13), 61(12), 58(11), 56(42), 55(15).

Degradation of **6** *to* 2-*methyl*-1d-1,4-*butanediol diacetate* (**11**). Acetylated **2** (183 mg) was hydrogenated as described above to give the crude hydrogenolysis product (160 mg) which was used in the next step without further purification. A soln of this material in 2N H_2SO_4 (4 ml) was heated on a steam bath for 2 hr. The hydrolysis mixture was cooled, and the pH was adjusted to 6·0 with Na_2CO_3 (500 mg). A soln of $NaBD_4$ (100 mg) in H_2O was added, and the mixture was stirred at room temp for 1 hr. Additional $NaBD_4$ (50 mg) was added, and stirring was continued for 2 hr. The soln was cooled, acidified to pH 1 with 10N H_2SO_4, saturated with NaCl, and extracted with EtOAc. The extract was dried (Na_2SO_4), concentrated, taken up in $CHCl_3$, filtered, and reconcentrated.

Acetylation of the $CHCl_3$ solubles was followed by chromatography on silica gel (3·3 g). Elution with $CHCl_3$ gave **11** (11 mg) which exhibited a single spot on TLC [$CHCl_3$-acetone (1:1), I_2 vapor] corresponding to **9**: IR ($CHCl_3$) 1735 cm^{-1}. NMR ($CDCl_3$) δ 0·95 (3H, d, $J = 6·5$ Hz), *ca* 1·7 (m), 2·02 (3 H, s), 2·03 (3 H, s), 3·91 (1 H, bd, $J = 6$ Hz) 4·11 (2 H, t, $J = 6·5$ Hz); GLC-MS of the center of the major peak eluted at 92° showed *m/e* (%) 190 ($M^+ + 1$; 1), 115(7), 103(6), 101(9), 100(7), 98(6), 87(8), 86(30), 85(13), 74(12), 73(6), 72(13), 70(18), 69(100), 68(11), 61(13), 57(14), 56(40), 55(16).

Detection of pinnatanine (**1**) *and oxypinnatanine* (**2**) *in Hemerocallis fulva.* Defatted seed meal (7 g) from *H. fulva* was extracted as for *S. pinnata* and the water-soluble portion applied to a 5 × 100 cm column of Sephadex G-10. The column was eluted with H_2O at a flow rate of 75 ml/hr, and 25-ml fractions were collected. Fractions 40–43 contained **1** as the sole ninhydrin reactive compound as shown by paper chromatography [n-BuOH-EtOH-H_2O (4:1:4)] and NMR. Fractions 33–35 were combined, concentrated, and applied to a column of AG 1-X4 anion exchange resin (acetate form). Ninhydrin-positive fractions eluted with H_2O (50 ml) were combined decolorized, and crystallized from EtOH to give **2**.

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