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THE MICROBIOLOGICAL TRANSFORMATION OF PARTHENIN BY *BEAUVERIA BASSIANA* AND *SPOROTRICHUM PULVERULENTUM**

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Key Word Index—*Beauveria bassiana*; *Sporotrichum pulverulentum*; fungi, biotransformation; *Parthenium hysterophorus*; sesquiterpene lactone; parthenin.

Abstract—Incubation of parthenin (1,6β-dihydroxy-4-oxo-10αH-ambrosa-2,11(13)-dien-12-oic acid-γ-lactone) with the fungus *Beauveria bassiana* affords the C-11 reduction product, 1,6β-dihydroxy-11β-methyl-4-oxo-10-αH-ambrosa-2-ene-12-oic acid-γ-lactone. A C-11 hydroxylation product, 1,6β,11α-trihydroxy-11β-methyl-4-oxo-10αH-ambrosa-2-ene-12-oic acid-γ-lactone was obtained from the broth of the fungus *Sporotrichum pulverulentum* using the same substrate.

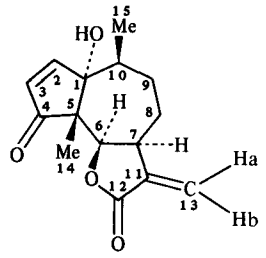
INTRODUCTION

In a recent communication we ascribed antiamoebic activity to parthenin (**1**) [2] which is the major sesquiterpene lactone of the noxious weed *Parthenium hysterophorus*. Earlier, parthenin had been found to be of interest for its anticancer and antigenic properties [3, 4]. This bioactive molecule in which the stereochemistry of the carbon skeleton has been rigorously established [5, 6], is a suitable starting point for transformation directed at structural correlations and structure–activity relationships. A study was initiated in our laboratory to prepare as many derivatives as possible and subjecting them empirically to *in vitro* antiamoebic and immunological tests. For this, a number of derivatives were prepared, principally by addition of various nucleophiles at C-2 or C-13 or at both the sites (unpublished results). As an extension to our chemical modification work, microbial transformations appeared to be the most suitable method for introducing new substituents onto the carbon skeleton of the sesquiterpene lactone without greatly disturbing the chromophores. The biotransformation of **1** by fungi has not been investigated previously.

RESULTS AND DISCUSSION

Separate incubation of **1** with *B. bassiana* and *S. pulverulentum* gave unidentical metabolites in 37 and 32% yields respectively. The structures of these metabolites and their dehydrated products were established by comparison of their spectroscopic data with that of parthenin.

Metabolite **2** obtained with *B. bassiana*, analysed for $C_{15}H_{20}O_4$ ($[M]^+$ at *m/z* 264) suggesting that the substrate had been reduced selectively. As its IR spectrum showed the presence of both ester and α,β-unsaturated carbonyls (1765 and 1710 cm^{-1} respectively), reduction had occurred to either of the endo- or exo-double bond. The shift in the absorption of ester carbonyl to shorter wavelength and absence of methylene stretchings from that of **1** (1736 cm^{-1}, 1652 and 1646 cm^{-1}) determined the site of the reduction. This was further confirmed from its ^1H NMR where doublet signals ($J = 2$ Hz) at δ 5.60 and



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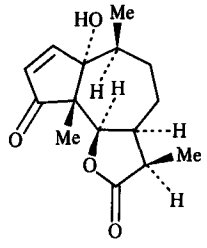

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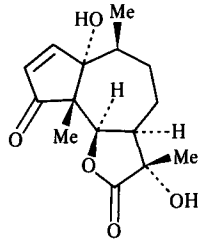

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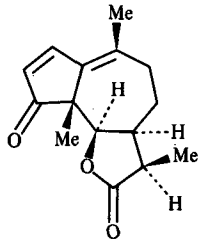

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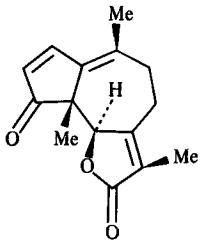

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*Part 5 in the series 'Plant-based antiamoebic drugs'. For part 4 see ref. [1].

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6.16 assigned to H-13a and H-13b in **1** were missing. Instead, a doublet at δ1.32 integrating for three protons appeared which was conveniently assigned to a C-13 methyl group. From the coupling constant ($J = 8$ Hz) this methyl group was further assigned an equatorial β-configuration. As the J value (8 Hz) for H-6 remained unchanged, an axial α-configuration was assigned to H-7.

Further evidence for the structure of **2** was provided from a study of its dehydrated derivative (**4**) obtained by BF_3–ether reaction. The latter gave an extended conjugated absorption peaks for cyclopentanone in UV (λ_{max} 290 nm) and IR (ν_{max} 1695 cm^{-1}).

The metabolite (**3**) obtained on incubation with *S. pulverulentum*, analysed for $C_{15}H_{20}O_5$ ($[M]^+$ at m/z 280) and suggested the addition of one molecule of water onto the double bond of the substrate. Its IR spectrum showed the presence of a saturated ester carbonyl (1770 cm^{-1}) absorption as for **2**. The presence of a C-13 methyl as a singlet in its ^1H NMR at δ1.30 and the appearance of C-2 and C-3 doublet signals at δ7.68 and 6.10, respectively, further proved the site of hydration. The J value (8 Hz) for H-6 established the α-configuration to C-7 proton. As for **2**, the dehydration with BF_3–ether conclusively proved its structure. The dehydrated derivative (**5**) thus obtained showed ester carbonyl absorption at 1735 cm^{-1} in its IR and three methyl singlets at δ1.30 (C-14), 1.96 (C-13) and 2.03 (C-15) in its ^1H NMR. Thus, fermentation with *S. pulverulentum* gave the hydrate of the Δ^{11}-double bond.

EXPERIMENTAL

Isolation and characterization of parthenin. Parthenin (**1**) used in the study was isolated from the $CHCl_3$ extract of *Parthenium hysterophorus* L. grown wild at our campus (collected at the flowering stage during August 1981) and characterized as reported previously [5, 6].

Incubation of parthenin with Beauveria bassiana *and* Sporotrichum pulverulentum. The fermentation broths of *B. bassiana* and *S. pulverulentum* were prepd from ten-days-old subcultures (maintained on potato dextrose agar, PDA, slants) in modified Richard's medium [7] which was prepd by dissolving KNO_3 (10 g), KH_2PO_4 (2.5 g), $MgSO_4$ (1.5 g), glucose (40 g), yeast extract (2 g) in double distilled H_2O (1 l). The medium was sterilized for 15 min at 120°, 15 lb/in^2 pressure. Both the inoculated media (200 ml) in shake flasks of 750 ml capacity were incubated for 3 days on a rotary shaker at 230 rpm with 1 inch throw at 25±1°. Dry powder **1** (1,6β-dihydroxy-4-oxo-10αH-ambrosa-2,11(13)-dien-12-oic acid-γ-lactone, 0.2 g) was added to each flask after 3 days of growth. The flasks were further incubated for 4 days. Samples (10 ml) were drawn aseptically to monitor the progress of the conversion. On completion of the incubation period, broths were centrifuged (400 rpm) for 10 min. The supernatant soln was sepd from mycelia in each flask and extracted with Et_2O (3 × 100 ml). The extract was dried and the solvent evapd. The residues from both the broths were separately chromatographed on silica gel to remove the unchanged parthenin. Elutions were done with mixts of EtOAc and C_6H_{14}. The crude conversion products thus obtained were recrystallized with EtOAc–petrol (60–80°) mixt. 1,6β-Dihydroxy-11β-methyl-4-oxo-10αH-ambrosa-2-ene-12-oic acid-γ-lactone (**2**, 75 mg, 37% yield after recryst.), mp 170–172°; λ_{max}^{MeOH} 215 nm (log ε 5.02); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH st), 2925, 1765 (ester C=O), 1710 (α,β-unsatd C=O), 1590 (C=C st), 1160 (OH bending) and 965; ^1H NMR (60 MHz, $CDCl_3$): δ1.12 (3H, *d*, $J = 8$ Hz, C-15), 1.30 (3H, *s*, C-14), 1.32 (3H, *d*, 8 Hz; C-13), 2.00 (4H, *br m*, C-8 and C-9), 2.28 (1H, t, $J = 4$ Hz, C-10), 2.36 (1H, *d*, $J = 8$ Hz, C-11), 2.58 (1H, *m*, C-7), 2.65 (1H, *br s*, collapsing on D_2O exchange, OH) 5.10 (1H, *d*, $J = 8$ Hz, C-6), 6.08 (1H, *d*, $J = 6$ Hz, C-3), 7.64 (1H, *d*, $J = 6$ Hz, C-2); EIMS m/z (rel. int.): 264 $[M]^+$ ($C_{15}H_{20}O_4$) (6.5), 246 (14.6), 20 (100).

Dehydration of metabolite (**2**) (50 mg) with BF_3–Et_2O drops at room temp. and usual work-up afforded the dehydrated product **4**, mp 163–164°, λ_{max}^{MeOH} 290 nm (log ε 5.12), ν_{max}^{KBr} cm^{-1}: 2900, 1765, 1695 (extended conjugated α,β-unsatd C=O), 1550 and 965; ^1H NMR ($CDCl_3$): δ1.28 (3H, *s*, C-14), 1.30 (3H, *d*, $J = 4$ Hz, C-13), 2.05 (3H, *s*, C-15), 2.28 (4H, *br m*, C-8 and C-9), 2.40 (1H, *d*, $J = 8$ Hz, C-11), 2.62 (1H, *m*, C-7), 5.18 (1H, *d*, $J = 8$ Hz, C-6), 6.12 (1H, *d*, $J = 6$ Hz, C-3), 7.96 (1H, *d*, $J = 6$ Hz, C-2), EIMS (rel. int.): 246 $[M]^+$ ($C_{15}H_{18}O_3$) (5.7).

Metabolite of *S. pulverulentum*, 1,6β,11α-trihydroxy-11β-methyl-4-oxo-10-αH-ambrosa-2-ene-12-oic acid-γ-lactone (**3**, 68 mg, 32% yield after recryst.), mp 177–178°; λ_{max}^{MeOH} 215 nm (log ε 4.80); IR ν_{max}^{KBr} cm^{-1}: 3380 (OH st), 2920, 1770 (ester C=O), 1715 (α,β-unsatd C=O), 1596 (C=C), 1150 and 1010 (OH bending) and 965; ^1H NMR ($CDCl_3$): δ1.14 (3H, *d*, $J = 8$ Hz, C-15), 1.27 (3H, *s*, C-14), 1.30 (3H, *s*, C-13), 2.18 (4H, *br m*, C-8 and C-9), 2.26 (1H, *t*, $J = 4$ Hz, C-10), 2.64 (1H, *m*, C-7), 3.32 (2H, *br s*, collapsing on D_2O exchange, OH), 5.18 (1H, *d*, $J = 8$ Hz, C-6), 6.10 (1H, *d*, $J = 6$ Hz, C-3), 7.68 (1H, *d*, $J = 6$ Hz, C-2); EIMS m/z (rel. int.) 280 $[M]^+$ ($C_{15}H_{20}O_5$) (5.3), 244 (33.6), 20 (100).

Dehydration of metabolite (**3**) (50 mg) with BF_3–Et_2O drops at room temp. and usual work-up afforded the dehydrated product **5**, mp 156–157°, λ_{max}^{MeOH} 215 and 290 nm (log ε 4.58, 4.56), IR ν_{max}^{KBr} cm^{-1}: 2910, 1735 (α,β-unsatd ester C=O), 1700 (α,β-unsat (C=O), 1555 and 965; ^1H NMR ($CDCl_3$): δ1.30 (3H, *s*, C-14), 1.96 (3H, *s*, C-13), 2.03 (3H, *s*, C-15), 2.36 (4H, *br m*, C-8 and C-9), 5.23 (1H, *s*, C-6), 6.04 (1H, *d*, $J = 6$ Hz, C-3), 7.96 (1H, *d*, $J = 6$ Hz, C-2); EIMS m/z (rel. int.): 244 $[M]^+$ ($C_{15}H_{16}O_3$) (9.7).

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