Two New Phytosterols from *Ailanthus altissima* (Mill) Swingle

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Abstract

A chloroform extract of the seeds of *Ailanthus altissima,* after silica gel column chromatography, gave two new sterols, named ailanthusterol A and ailanthusterol B. Their structures have been established as stigmast-5, 20(21)-diene-3β-ol and stigmast-5-ene-3β-21-diol on the basis of spectral data analysis and chemical reactions

INTRODUCTION

Ailanthus altissima (Mill) Swingle (Simarubaceae), Syn. *A. sladulosa* a 'Tree of Heaven' native to China and Japan is found in the hills of northern India up to an altitude of 2,400 m. Its bark possesses astringent, antispasmodic, anthelmintic, parasiticide and narcotic properties and is useful in diarrhoea and dysentery. Its other parts are recommended for different body disorders (The Wealth of India, 1985). Seeds (0.6 x 6.25 cm) are compressed, having membranous testa and scanty albumen, adhering to the testa with flat cotyledons (Kirtikar and Basu, 1191).

MATERIALS AND METHODS

Plant Material

Seeds (900g) of *A. altissima* were collected from the forests of Solan (H.P).

Extraction

The dried and powdered seeds were extracted with $CHCl_3$ in a Soxhlet apparatus. The extract was concentrated under reduced pressure to get a dark brown viscous semi-solid mass.

Isolation of Chemical Constituents

The concentrated extract was dissolved in the minimum amount of MeOH and adsorbed on silica gel to form a slurry. The slurry was air-dried and subjected to Si-gel column chromatography prepared in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol, in order of increasing polarity, to isolate the following compounds:

Ailanthusterol A (AA-1)

Elution of the column with petroleum ether (fraction 1-7) furnished a colourless amorphous powder of AA-1; recrystallized from $CHCl_3$ -MeOH (1:1), 0.78 g (0.08% yield), m.p. 271-272ºC, $[\alpha]_D^{30}$ = +1.47 (C 0.6, AcOH).

1. IR γ_{max} (KBr): 3425, 2910, 1630, 1598, 1460, 1380, 1365, 1160, 1070, 1020 $cm^{-1.}$

2. ^{1}H NMR (60 MHz, DMSO-d_6): δ 5.30 (1H, m, H-6), 4.77 (2 H, bm, H_2-21), 3.50 (1H, brm, w 1/2 = 9.5 Hz, H-3β), 0.96 (3H, brs, Me-19), 0.90 (3H, d, *J* = 6.0 Hz, Me-29), 0.85 (3H, d, *J* = 6.0 Hz, Me-26), 0.70 (3H, d, *J* = 6.0 Hz, Me-27), 0.63 (3H, brs, Me-18).

3. EIMS *m/z* (rel. int.): 412 [M]+ ($C_{29}H_{48}O$) (1.1), 394 (7.4), 379 (2.4), 273 (2.3), 255 (4.1), 213 (5.3), 201 (1.1), 192 (4.1), 174 (2.1), 164 (3.1), 160 (3.9), 152 (4.2), 146 (5.9), 144 (7.0), 135 (5.3), 132 (4.1), 124 (5.3), 120 (5.1), 118 (3.9), 108 (5.9), 106 (6.6), 95 (8.3), 83 (5.1), 81 (11.0), 72 (6.5), 69 (8.0), 57 (10.4).

4. Acetylation of AA-1: COMPOUND AA-1 (15 mg) was treated with AC_2O (3 mL) and pyridine (1 mL) at room temperature overnight. Water (10 mL) was added and the

reaction mixture extracted with $CHCl_3$. The $CHCl_3$-layer was washed with water, dried over Na_2SO_4 and evaporated to obtain the monacetyl product (AA-1a), m.p 146-147º, IR γ_{max} 1725cm^{-1}.

5. Oxidation of AA-1: Compound AA-1 (15 mL) was dissolved in Me_2CO (10 mL) and freshly prepared Jones reagent was added dropwise till the persistence of a brown colour at 4ºC. The reaction mixture was left at 25ºC for 2 hours, water (10 mL) added and worked up as usual to obtain the 3-oxo derivative (AA-1b) m.p. 133-134º, IR γ_{max}: 1710 cm^{-1}.

Ailanthusterol B (AA-2):

Elution of the column with petroleum ether (fraction 8-12) gave colourless beads of AA-2, recrystallized from $CHCl_3$-MeOH (1:1), m.p. 127-128º, $[\alpha]_D^{30}$ = -7.5 (C 1.37, $CHCl_3$).

1. UV λ_{max}: 212 nm (log ε 5.7).

2. IR γ_{max} (KBr): 3430, 2920, 2840, 1590, 1455, 1390, 1360, 1310, 1045 cm^{-1}.

3. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.36 (1H, d, *J* = 5.5 Hz, H-6), 3.52 (1H, brm, w 1/2 = 16.50 Hz, H-3α), 1.20 (3H, brs, Me-21), 1.00 (3H, brs, Me-19), 0.92 (3H, t, *J* = 6.5 Hz, Me-29), 0.86 (3H, d, *J* = 6.5 Hz, Me-26), 0.80 (3H, d, *J* = 6.5 Hz, Me-27), 0.68 (3H, brs, Me-18).

4. EIMS *m/z* (rel. int.): 430 $[M]^+$ ($C_{29}H_{50}O_2$) (N.O), 412 (3.8), 394 (7.1), 379 (6.1), 326 (5.3), 301 (5.8), 273 (3.2), 271 (3.6), 255 (7.8), 231 (2.4), 213 (8.6), 198 (4.3), 160 (8.1), 157 (8.9), 174 (1.5), 144 (12.3), 134 (7.3), 124 (2.4), 106 (14.9), 94 (14.5), 83 (10.1), 81 (12.5), 72 (9.7), 57 (16.0), 55 (21.6), 43 (27.0),

5. Acetylation of AA-2: Compound AA-2 (25mg) was acetylated with Ac_2O (5 mL) and pyridine (1 mL) at room temperature for 24 hours. Water (20 mL) was added and the reaction mixture extracted with $CHCl_3$ (3 x 10 mL). The organic phase was washed with H_2O, dried over Na_2SO_4 and evaporated to get the monoacetyl product (AA-2a), m.p. 114-115º, IR γ_{max}: 3400, 1725 cm^{-1}.

6. Jones oxidation of AA-2: Compound AA-2 (10 mg) was treated with a mixture of conc. H_2SO_4 (2 mL) and CrO_3 in acetone (5 mL) at 0º for 2 hours. Water (10 mL) was added and the reaction mixture extracted with $CHCl_3$ (3 x 10 mL). The organic phase was washed with H_2O (3 x 10 mL) dried over Na_2SO_4 and evaporated to get the 3-oxo derivative (AA-2b), m.p. 91-93º, IR γ_{max}: 3400, 1705 cm^{-1}.

RESULTS AND DISCUSSION

Compound AA-1

Compound AA-1, namely ailanthusterol A, was obtained as a colourless amorphous powder from petroleum ether eluents. It gave a positive Liebermann-Burchard test and showed characteristic IR absorption bands for hydroxyl groups (3425 cm^{-1}) and unsaturation (1630, 1598 cm^{-1}). Its mass spectrum showed a molecular ion peak at *m/z* 412 corresponding to a steroidal molecular formula $C_{29}H_{48}O$. The important ion peaks observed at *m/z* 394 [M-H_2O], 379 [394-Me], 273 [M-$C_{10}H_{19}$, SC], 255 [273-H_2O] and 213 [255-ring D], suggested that the compound possessed a C_{10}-unsaturated side chain and a hydroxy group and olefinic linkage in the steroidal carbon framework. The ion fragments at *m/z* 72 [$C_{1,10}$-$C_{4,5}$ fission]$^+$, 201 [M-71-SC]$^+$, 106 [124 ($C_{6,7}$-$C_{9,10}$ fission)-H_2O]$^+$, 120 [138 ($C_{7,8}$ -$C_{9,10}$)-H_2O]$^+$, 135 [M-138-SC]$^+$, 83 [$C_{2,3}$-$C_{5,10}$ fission]$^+$ and 69 [83-CH_2]$^+$ supported the presence of the hydroxyl group in ring A, placed at C-3 on biogenetic analogy and the olefinic linkage at C-5. The saturated nature of ring C was inferred from the ion peaks appearing at *m/z* 146 [164($C_{8,14}$ - $C_{9,11}$)-H_2O]$^+$ 160 [178 ($C_{8,14}$-$C_{11,12}$)-H_2O]$^+$, 174 [192($C_{8,14}$ -$C_{12,13}$)-H_2O]$^+$, 108 [M-164-SC]$^+$, 95 [M-178-SC]$^+$ and 81 [M-192-SC]$^+$.

The ^{1}H NMR spectrum of AA-1 displayed one-proton multiplet at δ 5.30 assigned to H-6. A two-proton multiplet at δ 4.77 was due to C-21 methylene groups. A broad multiplet at δ 3.50 (w 1/2 = 9.5 Hz), integrating for one proton, was ascribed to C-3 β-proton. Two three-proton signals at δ 0.96 and 0.63 were attributed correspondingly to C-

19 and C-18 methyl protons. Three doublets at δ 0.90, 0.85 and 0.70, each integrating for three protons and with a coupling constant of 6.0 Hz, were accounted to C-29 (primary) and C-27 and C-26 secondary methyls, respectively. The appearance of all the methyls in the range δ 0.96-0.63 attested to their location on the saturated carbons Acelyation of AA-1 yielded a monoacetyl product AA-1a. Treatment of AA1 with Jones reagent produced a 3-oxo compound (AA-1b), which responded to Zimmermann's test positively, thus confirming the presence of a hydroxyl group at C-3.

On the basis of these findings the structure of ailanthusterol A (AA-10 has been elucidated as stigmast-5, 20(21)-diene-3 β-ol. This is a new natural sterol containing a C-21 unsaturated methylene group.

Compound AA-2

Compound AA-2, namely ailantusterol B, was obtained as colourless beads from petroleum ether eluents. It showed a positive Liebermann-Burchard test and characteristic IR absorption bands for a hydroxyl group (3430 cm^{-1}), unsaturation (1590 cm^{-1}) and a gem-dimethyl/isopropyl group (1390, 1360, 1310 cm^{-1}). The electron impact mass spectrum showed the presence of diagnostically important peaks at *m/z* 412 [M-H_2O], 394 [M-2 x H_2O], 157 [$C_{10}H_{21}O$, side chain, SC]$^+$, 273 [M-SC], 255 [273-H_2O]$^+$, 231 [273-ring D]$^+$, 213 [255-ring D]$^+$ and 198 [213-Me]$^+$ (Scheme AA-II). These fragments (Knight, 1967, Gupta et al., 1992; 1994) suggested that the sterol possessed a saturated C_{10}-side chain with an ethyl group placed on C-24, on the basis of biogenetic consideration, and with a hydroxyl group and an unsaturated stigmastane carbon framework of the steroidal nucleus with a hydroxyl group. In addition to these, peaks at *m/z* 72 [$C_{1,10}$-$C_{4,5}$ fission]$^+$, 124 (ring B cleavage) 55 (72-H_2O]$^+$, 106 [124-H_2O]$^+$, 120 [134-H_2O]$^+$, 146 [M-238 (ring C cleavage)-H_2O]$^+$ and 83. [$C_{2,3}$-C_5, -$C_{7,5}$ fission]$^+$, suggested the location of another hydroxyl group in ring A, which was placed at C-3 on biogenetic grounds, and the presence of a trisubstituted double bond at C-5. The ^{1}H NMR spectrum of AA-2 exhibited a one-proton downfield doublet at δ 5.36 (*J* = 5.5 Hz) assigned to H-6. A one-proton broad triplet at δ 3.52 with a half-width of 16.5 Hz is associated with the C-3 axial proton. A three proton broad signal at δ 1.20 was accounted to the C-21 methyl group attached to a C-20 tertiary carbon containing a hydroxyl group. Two tertiary methyl signals at δ 1.00 (Me-19) and 0.68 (Me-18), one three-proton triplet for a primary methyl at δ 0.92 (*J* = 6.5 Hz, Me-29) and two three-proton doublets at δ 0.86 (*J* = 6.5 Hz, Me-26) and 0.80 (*J* = 6.5 Hz, Me-27) were in perfect agreement with the stigmast-5-ene diol skeleton. The remaining methylene and methine protons resonated between δ 2.80-1.08. Appearance of all the methyl signals in the region δ 1.20-0.68 supported the existence of these groups on the saturated carbons. Acetylation of AA-2 with acetic anhydride-pyridine afforded a monoacetyl product (AA-2A). Jones oxidation of AA-2 formed a 3-oxo derivative which gave a positive Zimmermann's test for 3-keto sterols, thus supporting the 3-hydroxyl group in the compound (Barton and Mayo, 1954). On the basis of this chemical and spectral evidence the structure of ailanthusterol B (AA-2) has been established as stigmast-5-ene-3 β,21 β-diol. This is also a new sterol derivative containing one hydroxyl group in the carbocyclic framework and another in the side chain.

Literature Cited

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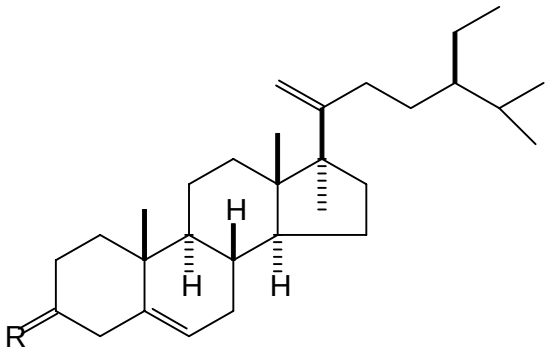
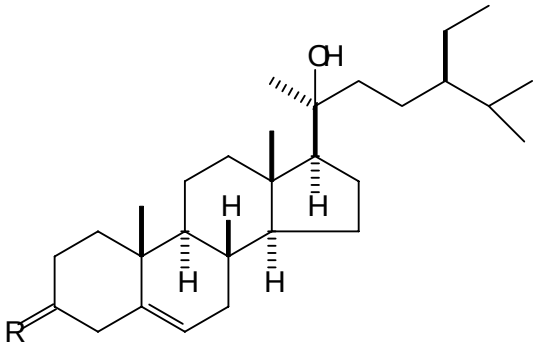
Figures



AA-1 : R=α-OH, H
AA-1a : R=α-Oac, H
AA-1b : R=O



AA-2 : R=β-OH, H
AA-2a : R=β-Oac, H
AA-2b : R=O