

AN ANTIBACTERIAL BIPHENYL DERIVATIVE AND OTHER CONSTITUENTS OF *ATYLOSIA TRINERVIA**[†]

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Key Word Index—*Atylosia trinervia*; Leguminosae; atylosol; lupenone; lupeol; sitosterol.

Abstract—The structure of a new antibacterial biphenyl, atylosol, from *Atylosia trinervia* was elucidated as 3-hydroxy-4-isopentenyl-5-methoxybiphenyl. In addition, lupenone, lupeol and sitosterol were also isolated.

INTRODUCTION

Atylosia trinervia Gamble cv Major Prain. (Leguminosae) is commonly distributed over Wester Ghats, Nilgiri hills and Atapadi hills of Malabar at an altitude of about 3000 m [1]. Only two species *A. goensis* and *A. scarabaeoides*, have been used in indigenous systems of medicine for rheumatism, biliousness and fever [2]. The alcoholic extract of *A. trinervia* has been recently reported to show antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* and to have a central nervous depressant effect [3], which prompted chemical investigation of this plant.

RESULTS AND DISCUSSION

In the present chemical and biological studies, the total alcoholic extract concentrate was acidified and extracted with CHCl_3 . The acid solution was worked up to give a basic fraction which showed CNS depressant activity. The solid obtained from the CHCl_3 fraction, was macerated with hexane, and was found to contain the antibacterial activity present in the plant. This residue showed four spots on TLC, which were designated as A, B, C and D in decreasing order of R_f values and the isolation of the pure substances was achieved by repeated CC separations on Si gel. The substance C, which exhibited activity at a concentration of 3.9 $\mu\text{g}/\text{ml}$, has been named atylosol.

Substance C (atylosol), colourless liquid, $\text{C}_{18}\text{H}_{20}\text{O}_2$ (M^+ , m/e 268), optically inactive, was indicated to be phenolic in nature and its IR spectrum showed absorptions at 3350 cm^{-1} for OH, a broad band at 1595 due to aromatic ring, 755, 700 for mono-substituted phenyl ring and 835 cm^{-1} for $-\text{CH}=\text{C}-$ in the molecule. The PMR spectrum exhibited two vinylic Me singlets at δ 1.28 and 1.51, two benzylic protons as a doublet centred at 2.96 ($J = 6\text{ Hz}$), one phenoxy Me singlet at 3.66, one OH proton centred at 4.40, one olefinic proton as a multiplet at 4.90, two *meta*-coupled aromatic protons as doublets ($J = 2\text{ Hz}$) centred at 6.06 and 6.20, and five protons as a sharp singlet at 7.13 ppm assignable to a phenyl ring.

Atylosol formed a monoacetyl derivative as a viscous

liquid, $\text{C}_{20}\text{H}_{22}\text{O}_3$ (M^+ , m/e 310) which showed a single peak on GLC. A band at 1750 cm^{-1} indicated a phenolic OAc in the molecule which was confirmed by the appearance of a singlet at 2.08 ppm in its PMR spectrum. In addition, both the *meta*-coupled aromatic protons were deshielded by *ca* 0.35 ppm to 6.40 and 6.48 ppm which indicated that phenolic OH was situated either *ortho* or *para* to these aromatic protons. Thus, atylosol contained a mono-substituted phenyl ring coupled to another phenyl ring which contained a OH, a OCH_3 , two *meta*-coupled protons and a 5 carbon side chain containing two vinylic Me groups.

The catalytic hydrogenation of atylosol acetate yielded a dihydro derivative, $\text{C}_{20}\text{H}_{24}\text{O}_3$. Its PMR spectrum displayed a doublet ($J = 7\text{ Hz}$) for two Me at 0.66 ppm, which confirmed them as vinylic in atylosol. In addition, a complex multiplet centred at 1.23 ppm assignable to two CH_2 and one methine protons. The two benzylic protons now appeared upfield as a triplet ($J = 6\text{ Hz}$) at 2.40 ppm indicating their allylic disposition and, therefore, the side chain was established as an isopentenyl group. Further proof of this was obtained by ozonolysis of atylosol and identification of acetone in the reaction mixture as its DNP derivative. Atylosol was, therefore, a biphenyl derivative having a hydroxyl, a methoxyl and an isopentenyl group attached to one ring.

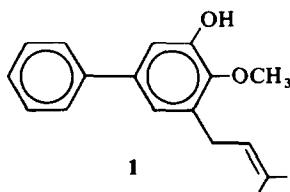
The acetyl dihydroatylosol on treatment with BBr_3 yielded a dihydroxy derivative as a viscous liquid, $\text{C}_{17}\text{H}_{20}\text{O}_2$, devoid of OAc and OMe groups. Its IR spectrum showed strong absorption at 3400 cm^{-1} for OH. This product on acetylation, yielded a diacetyl derivative whose IR spectrum showed a band at 1770 cm^{-1} and in its PMR spectrum, two singlets at 2.08 and 2.10 ppm were assigned to two aromatic acetoxy Me and a singlet at 6.70 ppm to the two *meta*-coupled aromatic protons. Atylosol on treatment with CF_3COOH in CHCl_3 [4] or HCl in AcOH [5], remained unchanged ruling out the *ortho* disposition of OH and isopentenyl side chain, but the treatment of acetyl atylosol with BBr_3 yielded a pyrano derivative as a liquid, $\text{C}_{17}\text{H}_{18}\text{O}_2$, M^+ m/e 254. Its IR spectrum did not show OAc absorption but a band at 3400 cm^{-1} showed the presence of OH. Its PMR spectrum exhibited a singlet at 1.28 ppm for a *geminal* Me on a carbon bearing an oxygen, a broad signal of a OH proton at 5.40 ppm and two *meta*-coupled

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aromatic protons as a singlet at 6.16 ppm in addition to other protons. Thus, the side chain was situated *ortho* to OMe.

The pyrano derivative yielded a monoacetate as a colourless liquid $C_{19}H_{20}O_3$, M^+ m/e 296 which showed a band at 1770 cm^{-1} for a phenolic OAc in the molecule. Its PMR spectrum exhibited a singlet for an acetoxy Me at 2.10 ppm and two *meta*-coupled aromatic protons at 6.30 ppm as a result of deshielding by 0.24 ppm.

Atylosol showed a positive Gibbs test due to the *para* position to OH being free. The optical inactivity of atylosol was indicative of the free *ortho* positions of the biphenyl chromophore which allowed free rotation of the rotamers. Taking into consideration the above data for the placement of the substituent groups, the structure of atylosol is assigned as 3-hydroxy-4-methoxy-5-isopentenyl-biphenyl (1).



Substance A (lupenone) mp 171°, $C_{30}H_{48}O$, M^+ , m/e 424, IR absorptions at 1710, 1630, 885 cm^{-1} , was reduced with NaBH_4 to lupeol, mp 201°, which was found to be identical with substance B (lupeol) $C_{30}H_{50}O$, M^+ , m/e 426 (monoacetate, mp 210°). Substance D, mp 139° was identified as sitosterol.

EXPERIMENTAL

The reported mps are uncorr. PMR spectra were recorded at 60 MHz in CCl_4 ; chemical shifts are expressed as δ values in ppm relative to TMS.

Isolation of the constituents. The powdered plant (12 kg) was extracted with EtOH and the extract was concd under red. pres. The concentrate was acidified and extracted with CHCl_3 . The acidic aq. soln which showed Dragendorff's positive spots on TLC was basified with NH_3 to pH 9.0 and repeatedly extracted with CHCl_3 . The CHCl_3 layer was washed with H_2O and concd to a dark green coloured residue (2.6 g) which showed CNS depressant activity (potentiation of barbital Na sleeping time in mice). It exhibited 3 spots, R_f 0.46, 0.53 and 0.56 on TLC (neutral Al_2O_3) in CHCl_3 -MeOH (9:1) with Dragendorff reagent and gave a blue colour with FeCl_3 . The CHCl_3 extract was concd to a dark green residue (180 g) and macerated with hexane to give a soluble fraction (125 g) which showed maximum antibacterial activity. It (50 g) was, therefore, chromatographed over Si gel (1 kg). The hexane eluates showing spot A were re-chromatographed over Al_2O_3 when the substance was eluted with hexane- $C_6\text{H}_6$ (1:1) and crystallized from EtOH, mp 171°, 1 g.

The subsequent hexane- $C_6\text{H}_6$ eluates showing spot B and C were rechromatographed over Al_2O_3 , when $C_6\text{H}_6$ eluates gave the pure substance B, crystallized from EtOH, mp 201°, 1.8 g. The CHCl_3 eluates contained substance C as the major spot and were concd and the residue (4.4 g) was acetylated. The oily reaction product was purified by chromatography over Si gel

and the hexane- $C_6\text{H}_6$ (1:3) eluates yielded substance C acetate which on saponification with dilute alkali gave substance C as a colourless liquid (2 g).

The $C_6\text{H}_6$ eluates were concd and the residue (2.13 g) on crystallization with EtOH yielded substance D as colourless needles, mp 139° (0.5 g).

Substance C (atylosol). It was obtained as a colourless viscous liquid and gave a blue colour with FeCl_3 . IR $\nu_{\text{max}}^{\text{Neat}} \text{cm}^{-1}$: 3350 (OH), 2950, 1615 (sh), 1595, 1575 (sh), 1450, 1425, 1345, 1150, 1120, 970, 920, 835, 755 and 700. PMR: δ 1.28, 1.51 (3H each, s, Me), 2.96 (2H, d, J = 6 Hz, Ar-CH₂-), 3.66 (3H, s, ArOMe), 4.40 (1H, b, $D_2\text{O}$ exchangeable, OH), 4.90 (1H, m, -CH=CH-), 6.06 and 6.20 (1H each, d, J = 2 Hz, Ar-H), 7.13 (5H, s, 5 \times Ar-H). MS m/e : 268 (M^+), 253, 225, 211, 181, 165, 152, 116, 91 and 77.

Atylosol acetate was obtained as a colourless liquid. On GLC (3% SE-30, 150 \times 4 cm SS column, 210°, flow pressure 31 psi) it showed a single peak having R_f 7.7 min. IR $\nu_{\text{max}}^{\text{Neat}} \text{cm}^{-1}$: 2900, 1750 (phenolic OAc), 1605, 1450, 1410, 1365, 1325, 1210, 1150, 1120, 1050, 970, 900, 758, 700 and 680. PMR: δ 1.30 and 1.55 (3H each, s, Me), 2.08 (3H, s, Ar-OCOMe), 3.1 (2H, d, J = 6 Hz, Ar-CH₂-), 3.73 (3H, s, ArOMe), 5.0 (1H, m, -CH=CH-), 6.40 and 6.48 (1H each, d, J = 2 Hz, Ar-H), 7.15 (5H, s, 5 \times Ar-H). MS m/e : 310 (M^+), 268, 253, 211, 181, 165, 152, 115 and 77.

Reduction of atylosol acetate. Atylosol acetate (140 mg) in EtOH was shaken with Pd/C (100 mg, 10%) in H_2 atmosphere for 1 hr. The reaction mixture was filtered and concd to a viscous residue (138 mg), R_f 0.8 ($C_6\text{H}_6$, AgNO_3 impregnated Si gel). IR $\nu_{\text{max}}^{\text{Neat}} \text{cm}^{-1}$: 2960, 1770, (OAc), 1590, 1455, 1415, 1365, 1330, 1205, 1135, 1050, 1010, 970, 900, 770 and 700. PMR: δ 0.66 (6H, d, J = 7 Hz 2 Me), 1.23 (3H, m, Ar-CH₂-CH₂-CH-), 2.06 (3H, s, ArOCOMe), 2.40 (2H, t, J = 6 Hz, Ar-CH₂-CH₂-), 3.7 (3H, s, ArOMe), 6.38 and 6.46 (1H each, d, J = 2 Hz, 2 \times Ar-H), 7.13 (5H, s, 5 \times Ar-H).

BBR₃ treatment of dihydroatylosol acetate. Dihydroatylosol acetate (100 mg) in dry CHCl_3 (10 ml) was cooled to -30° and BBR_3 (0.02 ml) was added. The reaction mixture was stirred at room temp. for 2 hr, diluted with H_2O and extracted with CHCl_3 which yielded a viscous residue (55 mg), R_f 0.3 ($C_6\text{H}_6$ -MeOH, 98:2). IR $\nu_{\text{max}}^{\text{Neat}} \text{cm}^{-1}$: 3400, 2960, 2940, 2860, 1600, 1460, 1365, 1125, 1005, 840, 770 and 700.

Acetylation of dihydromonohydroxyatylosol. The BBR_3 -derived product (50 mg) was treated with Ac_2O in $C_5\text{H}_5\text{N}$ (0.5 ml) and kept overnight at room temp. It was worked up to give a colourless viscous residue (55 mg), R_f 0.85 ($C_6\text{H}_6$ -MeOH, 98:2). IR $\nu_{\text{max}}^{\text{Neat}} \text{cm}^{-1}$: 3020, 2960, 2870, 1765, (OAc), 1600, 1460, 1365, 1200, 1110, 1020, 915, 895, 755 and 700. PMR: δ 0.66 (6H, J = 7 Hz, 2Me), 1.2 (2H, m, ArCH₂-CH₂-CH), 2.08 and 2.10 (3H each, s, ArOCOMe), 2.35 (2H, m, Ar-CH₂-CH₂), 6.7 (2H, s, 2 \times Ar-H), 7.2 (5H, s, 5 \times Ar-H).

Treatment of atylosol acetate with BBr₃. Atylosol acetate (100 mg) in dry CHCl_3 was reacted with BBr_3 (0.02 ml) as described above. This yielded a viscous residue (58 mg), R_f 0.15 ($C_6\text{H}_6$). IR $\nu_{\text{max}}^{\text{Neat}} \text{cm}^{-1}$: 3420, 3040, 2990, 2940, 1615, 1605, 1450, 1150, 1025, 920, 845, 770 and 700. PMR: δ 1.28 (6H, s, -OC(Me)₂), 1.61 (2H, t, J = 7 Hz, Ar-CH₂-CH₂), 2.46 (2H, t, J = 7 Hz, Ar-CH₂-CH₂), 5.4 (1H, d, $D_2\text{O}$ exchangeable, OH), 6.16 (2H, s, 2 \times Ar-H), 7.16 (5H, s, 5 \times Ar-H). MS m/e : 254 (M^+), 210, 198, 180, 140, 114.

Acetylation of dimethyl pyranoatylosol. The product (50 mg) in $C_5\text{H}_5\text{N}$ (0.5 ml) and Ac_2O (0.5 ml) were kept overnight at room temp. It gave a colourless oil (54 mg), R_f 0.5 ($C_6\text{H}_6$). IR $\nu_{\text{max}}^{\text{Neat}} \text{cm}^{-1}$: 3040, 2990, 2940, 1770, (OAc), 1600, 1420, 1370, 1325, 1210, 1140, 1120, 1030, 930, 900, 865, 770, 760, 700 and 680. PMR: δ 1.30 (6H, s, 2Me), 1.63 (2H, t, J = 7 Hz, Ar-CH₂-CH₂), 2.10 (3H, s, ArOCOMe), 2.5 (2H, t, J = 7 Hz, Ar-CH₂-CH₂), 6.40 (2H, s, 2 \times Ar-H), 7.2 (5H, s, 5 \times Ar-H). MS m/e : 296 (M^+), 254, 238, 210, 198, 140 and 114.

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