24-Methylenecholesterol in Tissue Cultures of *Holarrhena antidysenterica*

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Holarrhena antidysenterica Wall. (fam. Apocyanaceae) is known to be a rich source of a number of steroidal alkaloids [1]. In the course of our investigations on the isolation and characterisation of steroidal alkaloids and steroids from tissue cultures of the plant, we observed what appeared to be abnormal metabolism in regard to the steroidal components. This communication is concerned with the production and accumulation of 24-methylene cholesterol in substantial amounts in callus tissues raised from germinated seedlings of *H. antidysenterica*. 24-Methylene cholesterol is rarely present in the higher plants except in certain seed oils and pollen [2], although it is reported to occur widely in lower plants [3].

For the present investigation seedling callus tissues which have been under subculture for the past two years on a modified Murashige and Skoog's medium [4], supplemented with coconut milk, casein hydrolysate, inositol, adenine and 2,4-dichlorophenoxyacetic acid, were utilised for analysis. Oven dried tissue (100 g) was powdered and extracted first with methanol (cold) and then with methanol-benzene (50:50, reflux). The extracts were pooled and evaporated to dryness. The residue was dissolved in chloroform and extracted successively with 1 N HCl and 1 N NaOH for the removal of basic and acidic components. The chloroform layer consisting of neutral components was washed with water, dried over sodium sulphate and evaporated to dryness.

The residue was chromatographed on a silica gel column using benzene-ethyl acetate gradient. Fractions containing the sterol were pooled and further separated by preparative TLC, using silica gel G impregnated with 10% silver nitrate and chloroform. Three sterol bands were observed (a, b and c) which were eluted with chloroform-ethyl acetate (50:50).

The sterol in band 'c' on crystallisation from methanol gave 15 mg of a homogeneous product (mp 138 – 140 °C). The IR spectrum of the product showed strong absorption bands at 3450 cm^{-1} and 1060 cm^{-1} (OH), 888 cm^{-1} and 1640 cm^{-1} ($=CH_2$), 805 cm^{-1} and 845 cm^{-1} ($-C=CH-$). Its NMR spectrum confirmed the presence of a terminal methylene group (δ 4.7), and a vinyl proton (δ 5.3). Mass spectrum of the product gave a molecular ion ($M^{\oplus}$) at m/e 398, corresponding to the molecular formula $C_{28}H_{46}O$. The predominant fragmentation peaks at m/e 314 ($M-84$) and m/e 271 ($M-127$) were characteristic of a sterol having terminal methylene group in the side chain [5]. The IR spectrum of the compound was superimposable with that of an authentic sample of 24-methylene cholesterol. Its identity was further confirmed through its acetate (mp 134 – 135 °C).

Sterols from band 'a' were acetylated and separated on TLC using Anasil B and solvent system petroleum ether-ether (99:1). Comparison of R_f values with the authentic samples of sterol acetates showed that the major sterols were stigmasterol and sitosterol. However, trace amounts of campesterol and cholesterol were also found to be present. This was confirmed by a mass spectral analysis of the sample.

The synthesis of 24-methylene cholesterol in tissue cultures of *H. antidysenterica* and its absence in the intact plant [6] is rather unusual and investigation of the role of this sterol in tissue metabolism of higher plants would be of considerable interest and is being pursued.

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