A New 4β-Methyl-sterol from Marigold Flowers

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THE details of sterol biosynthesis are now under extensive investigation.[1] With the exception of 4β-methylcholesta-8, 24-dien3-β-ol[2] (isolated from rat skin after Triparanol treatment) all the methyl-sterols isolated up to now have the 4α-methyl group, which is apparently the last one to be removed from sterol precursors. I report the isolation of a new 4β-methylsterol from plant material.

Sterols and methyl-sterols were obtained from dry marigold flowers (*Calendula officinalis* L.) after solvent extraction, saponification, and column chromatography. They were separated on silver nitrate-impregnated silica gel. Together with the previously reported stigmasterol,[3] β-sitosterol, 28-isofucosterol (Δ^5-avenasterol) and traces of campesterol, 24-methylenecholesterol and cholesterol were identified. As minor constituents, two methyl-sterols were isolated in amounts of 20 and 4 mg. per kg. dry flowers, respectively.

The former had m.p. 165°, $[\alpha]_D$ +6°, its acetate m.p. 153°, $[\alpha]_D$ −21°. The mass spectrum of the sterol indicated the formula $C_{30}H_{50}O$ (M^+ 426) and a C_{10} side-chain with a 24-ethylidene group (fragments 328 and 285).[4] The Liebermann–Burchard reaction and the i.r. spectrum suggested that the second double bond was located at C-7. In the n.m.r. spectrum (acetate in CCl_4, 60 MHz.) chemical shifts of the 18-, 19-, and 29-methyl groups and of the proton at C-25 were identical with those of for stigmasta-7,24(28)-dien-3β-ol (Δ^5-avenasterol)[5], *i.e.* δ 0·53 s, 0·82 s, 1·57 d, *J* 7 Hz., and 2·7 septet, *J* 7 Hz. This supported the location of both double bonds[6] and indicated the configuration of the side-chain double bond to be identical with those of the avenasterols.[5] On the basis of the chemical shifts of the 18- and 19-methyl groups, the 14α-position for the additional methyl could be excluded.[6,7] Due to the distinct differences in optical rotations and slightly higher m.p., marigold methyl-sterol was thought to be an isomer of citrostadienol [4α-methylstigmasta-7,24(28)-dien-3β-ol][8] in respect to the configuration of the methyl group at C-4 [the avenasterol configuration of the side-chain 24(28) double bond can be predicted from molecular optical rotation differences for citrostadienol].

Hydrogenation of the marigold methyl-sterol in presence of acid and subsequent CrO_3–pyridine oxidation give a saturated 3-ketone. It had the positive Cotton effect $[\alpha]_{304} - [\alpha]_{265} = 500°$ (in MeOH) expected for a 4β-methyl-3-ketone, and no hemiketalisation was observed after addition of acid.[2] An acid isomerisation leading to a 4α-methyl-3-ketone (positive Cotton effect $[\alpha]_{305} - [\alpha]_{268} = 1600°$) unequivocally demonstrates that the marigold methyl-sterol is 4β-methylstigmasta-7,24(28)-dien-3-β-ol.

The second, minor, methyl-sterol has m.p. 151° and

formula $C_{29}H_{48}O$ (M^+ 412). Its mass and i.r. spectra suggest a C-24 methylene and it is thought to be 4β-methylergosta-7,24(28)-dien-3β-ol.

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