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TWO 3-OXO STEROIDS IN *THEA SINENSIS* SEEDS

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Key Word Index—*Thea sinensis*; Theaceae; 3-oxo steroids; spinasterone; 22,23-dihydrospinasterone; seeds.

Abstract—Spinasterone and 22,23-dihydrospinasterone were isolated from the seed oil of *Thea sinensis* which contains spinasterol and 22,23-dihydrospinasterol as the two major sterol constituents.

The sterol fractions separated from the unsaponifiable lipids of the seed oils from *Thea sinensis*, *Camellia japonica* and *C. sasanqua* have previously been studied and it has been shown that the fractions consist exclusively of Δ^7-sterols: spinasterol,* 22,23-dihydrospinasterol, avenasterol and 24-methyllathosterol, among which the former two constituted the major component sterols in each oil [1]. This paper describes a further study on the unsaponifiable lipid of *T. sinensis* seeds resulting in the identification of two 3-oxo steroids, spinasterone and 22,23-dihydrospinasterone, which are the ketones of the above two major sterols, respectively.

The unsaponifiable lipid (1.8 g) extracted and separated from *T. sinensis* L. seeds, which were courteously supplied by Professor M. Takido of this university, was separated into five major bands on Si gel TLC. The fraction (43 mg) recovered from the least polar band (R_f 0.56; cf. cholesterol, R_f 0.14) gave two peaks (RR_t 1.87 [A] and 2.11 [B]; cholesterol, RR_t 1.00) on GLC (OV-17 column). GC–MS (70 eV) of the faster eluted component (A, 63%) showed M^+ at *m/e* 410 which underwent loss of 139 mass units to give the ion at *m/e* 271 ($M^+ - C_{10}$ side chain). The other fragment ions at *m/e* 367 (M^+ – isopropyl group, mass 43) [2, 3], 298 ($M^+ - C_8H_{16}$, cleavage at C_{20}–C_{22} bond and one H) [4] and 269 (M^+ – side chain and 2H, base peak) indicated that the component was a $\Delta^{7,22}$-diunsaturated steroid with a C-24 ethyl group [5]. The absence of an ion at *m/e* 253 (M^+ – side chain and H_2O) suggested that C-3 was substituted with a carbonyl group which would not undergo fragmentation [6]. GC–MS of the slower eluted component (B, 37%) showed M^+, which formed the base peak, at *m/e* 412 accompanied with prominent fragment ions at *m/e* 271 ($M^+ - C_{10}$ side chain) and 229 (M^+ – side chain and 42 mass units). The large molecular ion indicated that it might be a Δ^7-monounsaturated steroid [5]. Moreover, the absence of the ion arising from M^+ – side chain and H_2O suggested the presence of a carbonyl group instead of a hydroxyl group at C-3 [6]. Reduction of the mixture with $LiAlH_4$ in dry Et_2O under N_2 and gentle reflux [7] yielded a mixture of spinasterol (RR_t 1.63) and 22,23-dihydrospinasterol (RR_t 1.86) which were identified by GLC and GC–MS. The two compounds, A and B, were therefore identified as the ketones of spinasterol and 22,23-dihydrospinasterol, i.e., spinasterone and 22,23-dihydrospinasterone, respectively. The identification of A and B was confirmed by the comparison of GLC and GC–MS data with those of authentic spinasterone and 22,23-dihydrospinasterone which were prepared from the corresponding 3β-alcohols by oxidation with CrO_3 in Py in the same way as described previously [8].

Spinasterone has previously been identified in *Samanea saman* [9] and *Careya arborea* [10], and 22,23-dihydrospinasterone in *Coccinia indica* [11], accompanied by the corresponding 3β-alcohols, spinasterol [9, 10] and 22,23-dihydrospinasterol [11], respectively. The co-occurrence of the 3-oxo steroids and the corresponding 3β-alcohols [1] was proved here in *T. sinensis* seeds, and this observation is of interest to considerations of sterol biogenesis. The occurrence of the 3-oxo-Δ^7-steroids is probable in other higher plants, including Theaceae, which contain exclusively Δ^7-sterols as the sterol constituents [1, 12].

EXPERIMENTAL

Spinasterol and 22,23-dihydrospinasterol used as the reference specimens were isolated from *T. sinensis* seed oil [1]. Most of the techniques used in this work have been described previously [1]. Spinasterone: MS (> *m/e* 100) *m/e* (rel. int.): 410 (20, M^+), 367 (24), 298 (18), 271 (34), 269 (100), 244 (13), 229 (21), 161 (13), 147 (22), 123 (24), 106 (19). 22,23-Dihydrospinasterone: MS *m/e* (rel. int.): 412 (100, M^+), 397 (30), 271 (85), 244 (22), 229 (53), 213 (15), 201 (10), 187 (11), 171 (11), 161 (18), 147 (16), 135 (20), 119 (27), 105 (26).

* Nomenclature: Spinasterol = 24*S*-ethyl-5α-cholesta-7,*E*-22-dien-3β-ol; 22,23-dihydrospinasterol = 24*R*-ethyl-5α-cholest-7-en-3β-ol; avenasterol = 24-ethyl-5α-cholesta-7,*Z*-24(28)-dien-3β-ol; 24-methyllathosterol = 24*RS*-methyl-5α-cholest-7-en-3β-ol (cf. ref. 13); spinasterone = 24*S*-ethyl-5α-cholesta-7,*E*-22-dien-3-one; 22,23-dihydrospinasterone = 24*R*-ethyl-5α-cholest-7-en-3-one.

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