A FACILE SYNTHESIS OF [3α-^{3}H]β-SITOSTEROL*

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ABSTRACT

Direct oxidation of the parent sterol using CrO_3 provided (24R)-24-ethyl-5-cholesten-3-one which on treatment with $NaBT_4$ gave [3α-^{3}H] (24R)-24-ethyl-5-cholesten-3β-ol. Purification at each stage afforded samples which were compared spectrally with the corresponding cholesterol series compounds.

INTRODUCTION

The plant sterols campesterol and β-sitosterol are less well absorbed than cholesterol by the mammalian intestine (1-5). Although the additional alkyl groups in the side chain of the plant sterols obviously influence their absorption, the mechanism of intestinal discrimination of absorbable and nonabsorbable sterols is not understood (1-5). Recently patients with the rare inherited lipid storage disease sitosterolemia with xanthomatosis have been shown to accumulate plant sterols (β-sitosterol [mg/dl mean±S.D.] [13±5] normal [<1.0] and campesterol [7.1±2.5] normal [<1.0]) and cholesterol in their plasma and tissue (6-10). Bhattacharyya and Connor have shown that the absorption of plant sterols in these patients is abnormal. Therefore, to study the metabolism of β-sitosterol in patients with this condition we have prepared radioactive (3α-^{3}H) β-sitosterol (Fig 1, IIIb) via $NaBT_4$ reduction of the intermediate (24R)-24-ethyl-5-cholesten-3-one (Fig 1, IIb).

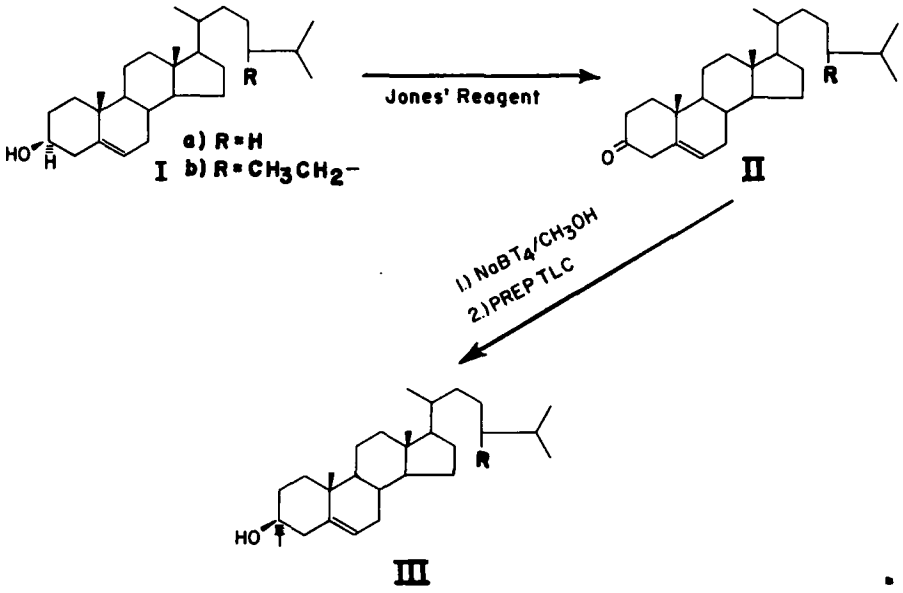

Fig.1 PREPARATION OF TRITIUM LABELED β-SITOSTEROL

METHODS

Melting points were determined on a Thermolyne apparatus (Thermolyne Corp., Dubuque, Iowa) model MP-12600, and are uncorrected.

TLC: β-Sitosterol and its corresponding intermediate Δ^5-3-one were separated on Silica gel G plates (Brinkmann, Westbury, N.Y.) (0.25 mm thickness) impregnated with $AgNO_3$ (7) (activated at 120° for $^1/_2$ hr and stored in a light-tight dessicator); solvent system: chloroform:acetone, 97:3 (v/v). Spots were made visible with rhodamine B, and areas corresponding to unsaturated and saturated sterol standards run on the same plate were scraped, extracted with diethyl ether and analyzed by GLC-MS.

GLC: The sterols as their TMSi (trimethylsilyl) derivatives were analyzed on a 6 ft. x 4 mm i.d. column packed with 3% QF-1 on 80/100 mesh gas Chrom Q (Applied Science Laboratories, State College, PA.); column temp. 270°, N_2 flow = 31 ml/min (Hewlett-Packard Model 7610 gas chromatograph, Palo Alto, CA.).

Mass spectra of the sterols were obtained with a Varian MAT CH-5 gas chromatograph-mass spectrometer (Varian Associates, Palo Alto, CA.) as described previously (7).

The radioactivity in aliquots of the material eluted from the different zones of the chromatoplate was measured with a Beckman LS 100C scintillation counter, using toluene phosphor as scintillation fluid. Under the

conditions employed, the counting efficiency was 47% for ^{3}H.

Radiochemical purity was determined after TLC by scraping the bands from the silica gel plate in sections and counting them using the liquid scintillation counter and by reverse radioisotope dilution analysis.

EXPERIMENTAL

Purification of β-Sitosterol: β-Sitosterol obtained from tall oil was dissolved in warm 2-propanol, filtered through a 0.2 mm filter and crystallized several times from 2-propanol-acetonitrile. The material melted at 129-139° and had trace contaminants. This was then further recrystallized several times and after final purification by argentation TLC in the solvent system: $CHCl_3$:$(CH_3)_2CO$, 97:3 (v/v) afforded 96% pure (GLC, Table 1) material which melted at 133-137° [lit mp 136-138°, $[\alpha]_D^{25} = -24°$(11)].

[Since β-sitosterol is structurally similar to cholesterol and had been found to be associated with the artifacts (epoxide formation) arising from its air oxidation, it is very difficult to get an extremely pure sample of this compound (12,13)].

Preparation of [3α-^{3}H] [(24R)-24-ethyl-5-cholesten-3β-ol) (Fig. 1,IIIb)].

β-Sitosterol(I, 9 mg; mp 133-137°, $[\alpha]_D^{25} = -24°$) in acetone (5 ml, distilled from $KMnO_4$) was cooled in ice (8-12) and while stirring, 10 µl of Jones' reagent (14) was added after 4 min followed by 1 ml methanol. Nitrogen gas was bubbled through all the solvents, reagents and reaction solution before and during the oxidation. After 6 minutes, the reaction mixture was diluted with diethyl ether and filtered through silica gel. Evaporation of the filtrate afforded crude (24R)-24-ethyl-5-cholesten-3-one (6 mg) (Fig. 1,IV), m/z 412. This crude material was applied to a 20% $AgNO_3$-silica gel G plate and developed with chloroform:acetone, 97:3 (v/v). The main band (R_f 0.78) was extracted with ethyl acetate and washed with dilute ammonium hydroxide to remove the rhodamine B used for visualization of the TLC plates. The Δ^5-3-one intermediate (after evaporation of the ethyl acetate extract) was then taken up in methanol/ethyl ether (2:1, 3 ml) containing $NaBT_4$ (25 MCi;) together with 6 mg of unlabeled $NaBH_4$. After stirring for $^1/_2$ hr, the reaction was stopped by the addition of dil CH_3COOH, solvents were removed in vacuo, and the residue was purified by preparative argentation TLC, solvent system $CHCl_3$:$(CH_3)_2CO$, 97:3 (v/v). Zonal scraping after using a thin-layer radiochromatogram scanner provided 3.5 mg of a chromatographically pure [3α-^{3}H] (24R)-24-ethyl-5-cholesten-3β-ol; R_f = 0.20, $CHCl_3$:$(CH_3)_2CO$, 97:3 (v/v), RRT = 2.94 min (as a TMSi derivative; RT of 5α-cholestane = 5.86 min) with a constant specific

activity of 5.3×10^6 dpm/mg, radiochemical purity 97.8%. The above compound appeared to be chemically and radiochemically homogeneous also by TLC with petroleum ether:acetone (70:30) and hexane:ethyl acetate (90:10).

The mass spectrum of the [3α-^{3}H] β-sitosterol (underivatized) was M^+, m/z 414 (80%) with ions at m/z 399 (26%, M^+-CH_3), 396 (38%, M^+-H_2O), 381 (28%, $M^+-H_2O-CH_3$), 329 (45%, $M^+-C_6H_{13}$), 273 (23%, M^+-S.C.), 253 (M^+-H_2O-S.C.), 43 (100%, the base peak) (S.C.=side chain).

RESULTS AND DISCUSSION

The parent sterols (R=H, cholesterol, R=C_2H_5, β-sitosterol) were oxidized using CrO_3 as reported by Djerassi, Engle and Bowers (14). The 3-oxo-5-ene structure (Fig. 1,II) was confirmed by GLC-MS and ultraviolet spectrophotometry. GLC-MS showed the presence of molecular ions at m/z 384 and 412 in the electron impact spectra of the cholesterol and β-sitosterol oxidation products. The presence of the 3-oxo-5-ene unit in each compound was established by the absence of the UV absorption maximum at 241 nm. After the addition of 1 drop of 1% KOH solution, UV absorption maximum of 241 nm was attained which was identical to that of 4-cholesten-3-one.

The subsequent reduction of the Δ^5-3-one intermediate with $NaBT_4$ gave radiochemically pure (97.5%) β-sitosterol. The labeled compound was stable for at least one month. In fact, it was administered to sitosterolemia subjects as a biogenetic precursor in a one month period; TLC controls showed that it was chemically and radiochemically unaltered.

TABLE 1: GLC AND TLC CHARACTERISTICS OF NEUTRAL STEROLS

Compound	TLC[a] R_f	GLC[b] RRT
Cholesterol	0.20	1.85
Campesterol	0.20	2.39
β-Sitosterol	0.20	2.94
Stigmasterol	0.20	2.61
Cholestanol (5α-cholestan-3β-ol)	0.28	1.87
Coprostanol (5β-cholestan-3α-ol)		1.47

[a]Argentation TLC, solvent system: $CHCl_3$:$(CH_3)_2CO$, 97:3 (v/v).
[b]Column, 3% QF-1, Temp. 270°, N_2 flow 31 ml/min Retention times of TMSi ethers relative to 5α-cholestane (RT = 5.86 min).

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