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The Isolation of Cholest-5-ene-3β,26-diol from Human Brain

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Cholest-5-ene-3β,26-diol, isolated from human brain, was further characterized by oxidation to 3-oxocholest-4-en-26-ol and to 3-oxocholest-4-en-26-oic acid. Identification was achieved by comparison (by t.l.c., g.l.c. and g.l.c.–mass spectrometry) with corresponding reference compounds derived from kryptogenin.

The presence of a cholest-5-ene-3β,26-diol‡ in normal and atherosclerotic human aortic tissue has been amply demonstrated (Brooks *et al.*, 1966; van Lier & Smith, 1967; Smith & van Lier, 1970; Fumagalli *et al.*, 1971), though the diol could not be detected in normal and experimental atherosclerotic aortas from a variety of animals (Kritchevsky *et al.*, 1970; Smith & Pandya, 1973). The sterol has also been isolated from commercial cholesterol that had originated from bovine brain and spinal cord (van Lier & Smith, 1971), but attempts to demonstrate its presence in brain and other tissues from humans have been unsuccessful (van Lier & Smith, 1969; Smith & Pandya, 1973). This apparent restriction of cholest-5-ene-3β,26-diol to the aorta in humans, its increase with the severity of atherosclerosis (Smith & van Lier, 1970; Fumagalli *et al.*, 1971) and its reported toxic effects (Biswas *et al.*, 1964; MacDougall *et al.*, 1965) have led to speculation about its role in atherogenesis (Kritchevsky *et al.*, 1970; Smith & van Lier, 1970; Fumagalli *et al.*, 1971; Smith & Pandya, 1973). We have examined two human brains and have isolated cholest-5-ene-3β,26-diol from both samples.

Experimental

Materials and general methods. Brains were obtained within 24h *post mortem*. Cholest-5-ene-3β,26-diol was prepared from kryptogenin (Scheer *et al.*, 1956); cholest-5-ene-3β,25-diol and (24-*RS*)-cholest-5-ene-3β,24-diol were gifts from Organon Laboratories Ltd., Newhouse, Lanarkshire, U.K. Cholesterol oxidase was given by Boehringer Corp. (London) Ltd., London W.5, U.K. Silica gel for dry column chromatography was obtained from Woelm Ltd., Eschwege, Germany: the chromatograms were developed with chloroform – ethyl acetate mixtures. T.l.c. was performed on Kieselgel HF_{254} with chloroform–ethyl acetate (3:1, v/v) as the mobile phase. G.l.c. was carried out at 282°C with a Pye–Unicam 104 instrument fitted with a glass column (5m × 3mm) packed with 1% SE-30 on Gas-Chrom Q (100–120 mesh). Combined g.l.c.–mass spectrometry was done with an LKB 9000 instrument fitted with a column (3m × 3mm) of 1% SE-30. The carrier gas was helium, the column temperature 270°C and the electron energy 70eV. Oxidations with cholesterol oxidase were done by incubation of the sterol (approx. 1mg in 100μl of acetone) with 0.5 unit of enzyme in 10ml of 50mM-NaH_2PO_4–Na_2HPO_4 buffer (pH7.0) for 3h at 37°C. 26-Hydroxycholest-4-en-3-one was oxidized by $CrCO_3$–H_2SO_4–water–acetone as described by Zaretskaya *et al.* (1968).

Isolation of cholest-5-ene-3β,26-diol. Brain I (male subject). The tissue (1.3kg) was homogenized in acetone (3 litres), the homogenate filtered through glass wool and the remaining solids were extracted twice with chloroform–methanol (2:1, v/v). After removal of solvents from the combined filtrates the residue was dissolved in chloroform, refiltered through Celite 535 and evaporated to dryness to yield the total lipid (120g). This was chromatographed on silica gel to give a fraction (1.2g) containing polar sterols and cholesterol. Further column chromatography followed by t.l.c. yielded material (2.3mg) co-chromatographing with cholest-5-ene-3β,26-diol (R_F 0.38).

Brain II (female subject). The tissue (1.2kg) was saponified (Smith *et al.*, 1973) in the presence of pyrogallol (5g) as an antioxidant, to yield 30g of non-saponifiable lipid. Cholesterol was largely removed by crystallization from chloroform–methanol. The remaining lipid (12g) was chromatographed on two successive 'dry columns' to give a fraction (345mg) containing polar sterols. T.l.c. gave 1.8mg of material corresponding to cholest-5-ene-3β,26-diol.

Results and discussion

The samples of cholest-5-ene-3β,26-diol (R_F 0.38) could be distinguished by t.l.c. from cholesterol

‡ The prefix 26 is used herein to designate either of the terminal methyl groups.

Table 1. *Comparison of g.l.c. results for derivatives of cholest-5-ene-3β,26-diol samples obtained from human brain and from kryptogenin*

Retention index values of samples from different sources, values rounded to the nearest 5 units. For details see the text.

Cholest-5-ene-3β,26-diol derivative	Brain I	Brain II	Kryptogenin
Cholest-5-ene-3β,26-diol bis(trimethylsilyl) ether*	3490	3490	3490
Cholest-5-ene-3β,26-diol diacetate	3635	—	3635
25-Acetoxycholest-4-en-3-one	3630	—	3625
Methyl 3-oxocholest-4-en-26-oate	—	3530	3525

* cf. Cholest-5-ene-3β,25-diol bis(trimethylsilyl) ether, 3440; (24*RS*)-cholest-5-ene-3β,24-diol bis(trimethylsilyl) ether, 3425.

Table 2. *Mass-spectrometric results for the derivatives of cholest-5-ene-3β,26-diol obtained from human brain*

Spectra were obtained by g.l.c.–mass spectrometry as described in the text. Ions below *m/e* 70 have been excluded. Relative intensities are given in parentheses. Identities of ions: (*a*), $M-CH_3$; (*b*), *M*−trimethylsilanol or acetic acid; (*c*), $M-CH_3-$trimethylsilanol or acetic acid; (*d*), *M*−2 acetic acid moieties; (*e*), *M*−side chain; (*f*), *M*−side chain-trimethylsilanol or acetic acid; (*g*), *M*−129; (*h*), *M*−123; (*i*), *M* − 42; (*j*), *M*−side chain−42.

	Ions (*m/e*)												
Derivative	$M^{+\cdot}$	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*		
Cholest-5-ene-3β,26-diol bis-(trimethylsilyl) ether	546 (8)	531 (2)	456 (22)	441 (7)	—	—	255 (9)	417 (23)	—	—	—	73 (100)	129 (87)
Cholest-5-ene-3β,26-diol diacetate*	—	—	426 (78)	411 (9)	366 (28)	—	255 (22)	—				81 (100)	158 (80)
26-Acetoxycholest-4-en-one	442 (36)	427 (2)	382 (3)	—	—	271 (9)	—	—	319 (10)	400 (9)	229 (24)	124 (100)	
Methyl 3-oxocholest-4-en-26-oate	428 (34)	413 (2)	—	—	—	271 (9)	—	—	305 (12)	386 (5)	229 (44)	88 (11)†	124 (100)

* The mass spectrum corresponded closely to that previously recorded in our laboratory for the diacetate derived from aortal diol (Steel, 1969).

† In all other spectra *m/e* 88 was <1% abundance.

(R_F 0.61), (24-*RS*)-cholest-5-ene-3β,24-diol (R_F 0.49) and cholest-5-ene-3β,25-diol (R_F 0.41). The sterol from brain I (m.p. 173–175°C) was analysed by g.l.c. as its bis(trimethylsilyl) ether and as its diacetate (m.p. 121–123°C) in comparison with the sterol derived from kryptogenin (Table 1). The remainder was incubated with cholesterol oxidase to give 26-hydroxycholest-4-en-3-one (R_F 0.54), which was acetylated and examined by g.l.c. All three derivatives of the cholest-5-ene-3β,26-diol isolated from brain I were almost identical in their g.l.c. properties and mass spectra with the analogous compounds prepared from the reference diol derived from kryptogenin (Tables 1 and 2). Notable in the mass spectra were ions *e*, *f* and *j* formed by cleavages involving loss of an oxygenated side chain. The presence of the Δ^5-steroid 3-trimethylsilyloxy grouping in the bis-(trimethylsilyl) ether was confirmed by the ion of *m/e* 417 (*g*) and the complementary ion *m/e* 129, arising by fission of the C-1–C-10 and C-3–C-4 bonds (Eneroth *et al.*, 1964; Brooks *et al.*, 1967; Diekman & Djerassi, 1967). The formation of the 4-en-3-one system by the enzyme to give 26-hydroxycholest-4-en-3-one was illustrated by the ions in the mass spectrum of the acetate at *m/e* 319 (*h*) and *m/e* 124 formed by cleavage of ring B, and *m/e* 400 (*i*) and *m/e* 229 (*j*) derived by elimination of C-2 and C-3 (Budzikiewicz *et al.*, 1964; Schubert *et al.*, 1969; Zaretskaya *et al.*, 1968).

The sample of cholest-5-ene-3β,26-diol from brain II was also analysed by g.l.c. as its trimethylsilyl ether (Table 1), then oxidized with enzyme to the corresponding 4-en-4-one. Further oxidation with CrO_3–H_2SO_4–water–acetone gave 3-oxocholest-4-en-26-oic acid, which was converted by CH_2N_2 into methyl 3-oxocholest-4-en-26-oate (R_F 0.76). The g.l.c. and mass-spectral properties of this ester were indistinguishable, under the conditions used, from those of

the corresponding compound made from kryptogenin (Tables 1 and 2). The formation of a carboxyl function confirmed that the side-chain hydroxyl group of the isolated diol was primary, whereas the ion at *m/e* 88 [$(CH_3CH_2CO_2CH_3)^{+\cdot}$], in the mass spectrum of the methyl ester, indicated the presence of an α-methyl substituent (Ryhage & Stenhagen, 1960; Zaretskaya *et al.*, 1968). Again the ion of *m/e* 124 and the ions *h*, *i* and *j* (*m/e* 305, 386 and 229 respectively) revealed the presence of a 4-en-3-one system in this derivative (Table 2).

The presence of cholest-5-ene-3β,26-diol at concentrations of 1.5 and 1.75 μg/g of brain tissue contrasts with a previous observation that, if the sterol was present, it did not exceed 0.1–1 μg/whole brain (van Lier & Smith, 1969). The formation of the diol from [4-^{14}C]cholesterol by liver mitochondria (Fredrickson & Ono, 1956; Danielsson, 1961; Mitropoulos *et al.*, 1972) and by human intimal tissue (Manzur & Chobanian, 1970) suggests that the human brain hydroxy sterol is produced enzymically.

The melting point of the isolated diol (173–175°C) was comparable with those reported for aortal cholest-5-ene-3β,26-diol (172.5–174°C and 174.5–176.5°C; van Lier & Smith, 1969) and for the (25*R*)-sterol derived from kryptogenin [177–178°C, Scheer *et al.* (1956); 173–175°C, Danielsson (1961); 175.5–176.5°C, Wachtel *et al.* (1968)]. The melting point of the diol diacetate (121–123°C), however, was considerably lower than that of the (25*R*) isomer (128–129°C) [literature m.p.: 128–129°C, Scheer *et al.* (1956), Wachtel *et al.* (1968); 130–131°C, Mitropoulos *et al.* (1972)]. A melting point of 127–128.5°C was recorded for the diacetate of a specimen of the aortal diol (van Lier & Smith, 1967). Schubert *et al.* (1969) converted 26-hydroxycholest-4-en-3-one, obtained by microbial oxidation of cholesterol, into cholest-5-ene-3β,26-diol and its diacetate (m.p. 171–173°C and 119–123°C respectively). X-ray studies have since shown this hydroxy-enone to be the (25*S*) isomer (Duchamp *et al.*, 1971). We are unable, on our present evidence, to assign the absolute configuration at C-25 in the cholest-5-ene-3β,26-diol isolated from human brain.

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