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Note

Configuration at C-25 and stereochemical purity of 26-hydroxycholesterol of natural origin

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26-Hydroxycholesterol (cholest-5-ene-3β,26-diol) has been repeatedly[1–3] isolated from normal and atherosclerotic human aortic tissue. Although its melting point and behaviour in thin-layer (TLC) and gas–liquid chromatography (GLC) are in agreement with data reported for (25*R*)-26-hydroxycholesterol derived from kryptogenin[4], some doubt has persisted with respect to its configuration at C-25 and its stereochemical purity. This was chiefly because of the lack of pure reference (25*S*)-26-hydroxycholesterol and of a method for resolving the C-25 epimers.

We have recently reported[5] the resolution of a synthetic mixture of (25*R*)- and (25*S*)-26-hydroxycholesterol achieved at the stage of the 3,26-diacetate by high-pressure liquid chromatography (HPLC) and leading to pure (25*S*)-26-hydroxycholesterol. The separation was performed by recycling on silica of high surface area.

These results prompted us to investigate 26-hydroxycholesterol from human aortic tissue by HPLC in order to assign unambiguously its C-25 configuration and stereochemical purity. In addition, 26-hydroxycholesterol (of unknown configuration at C-25) isolated[6] from air-aged cholesterol was similarly tested.

EXPERIMENTAL

Instrumental

HPLC was performed with a Waters Assoc. chromatograph equipped with a 6000A pump, a U6K injector, a refractometric detector and two columns (each 30 cm × 4 mm I.D.) packed with μPorasil. The mobile phase was *n*-hexane containing 2.5% (v/v) of ethyl acetate at a flow-rate of 1 ml/min and a pressure of 560 p.s.i.

Melting points were measured in open capillary tube and are uncorrected.

TLC was carried out on layers (200 × 200 × 0.25 mm) of Merck silica gel GF_{254}, with ethyl acetate–*n*-heptane (1:1, v/v) as developing solvent. Spots were visualized by spraying with 5% (w/v) phosphomolybdic acid in ethanol and heating.

Samples of 26-hydroxycholesterol

26-Hydroxycholesterol isolated from human aortas[2] and from air-aged cholesterol[6] were gifts from Dr J. E. van Lier, of the Département de Médecine Nucléaire et de Radiobiologie, Centre Hospitalier Universitaire, Sherbrooke, Quebec, Canada.

The 26-hydroxycholesterol derived from kryptogenin[4] was a gift from Dr E. Caspi, of The Worcester Foundation for Experimental Biology, Shrewsbury, Mass., U.S.A., and was considered as being optically pure.

Acetylation of 26-hydroxycholesterol

26-Hydroxycholesterol from human aorta or other source (1–2 mg) was acetylated (acetic anhydride and pyridine at room temperature), and the homogeneity of the resulting 3,26-diacetate was checked by TLC (R_F 0.9). It was then dissolved in 2.5% ethyl acetate solution in *n*-hexane (*ca.* 1 ml), and the solution, filtered is necessary, was injected into the HPLC system.

RESULTS

HPLC of 26-hydroxycholesterol 3,26-diacetate resulting from acetylation of 26-hydroxycholesterol from human aorta revealed (after seven recycles) the presence of two compounds (Fig. 1), which were collected. The less polar constituent (10% of the total acetylated material) was identical with the (25*S*)-26-hydroxycholesterol 3,26-diacetate described previously[5]. Identity was established by admixture with authentic (25*R*)- or (25*S*)-26-hydroxycholesterol 3,26-diacetate and HPLC. Only with the former epimer was resolution achieved after several recycles.

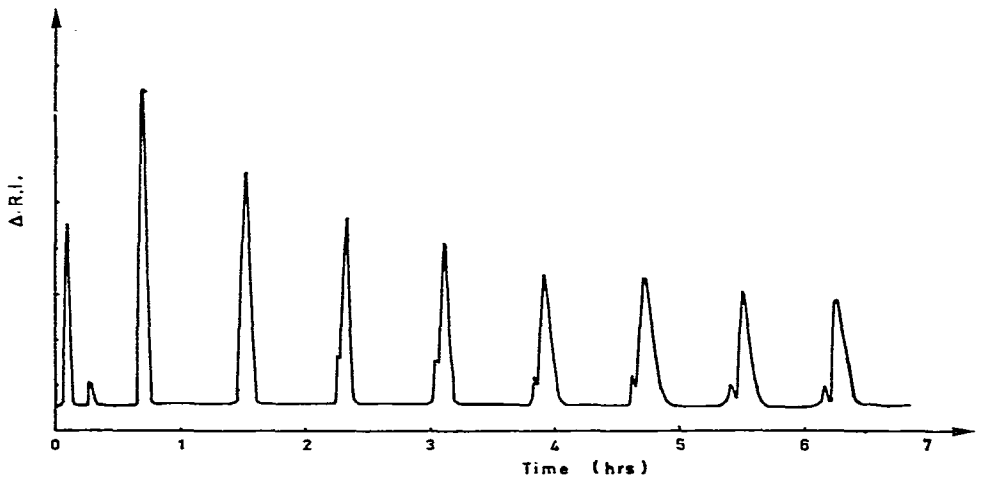

Fig. 1. HPLC of 26-hydroxycholesterol 3,26-diacetate from human aorta; after seven recycles, resolution is achieved. The less polar peak represents the 25*S*-epimer and the more polar one the 25*R*-epimer. The compounds were detected by refractometry.

The more polar constituent (90% of the total acetylated material) was identical with authentic (25*R*)-26-hydroxycholesterol 3,26-diacetate[4,5] according to melting point (128°) and mixed melting point. Further proof of identify was provided by admixture with either the 25*R*- or the 25*S*-epimer and HPLC as described above.

26-Hydroxycholesterol 3,26-diacetate prepared from 26-hydroxycholesterol derived from kryptogenin[4] was submitted to HPLC; the result was similar to that shown in Fig. 1, except the 25*S*-epimer represented only 3% of the total material.

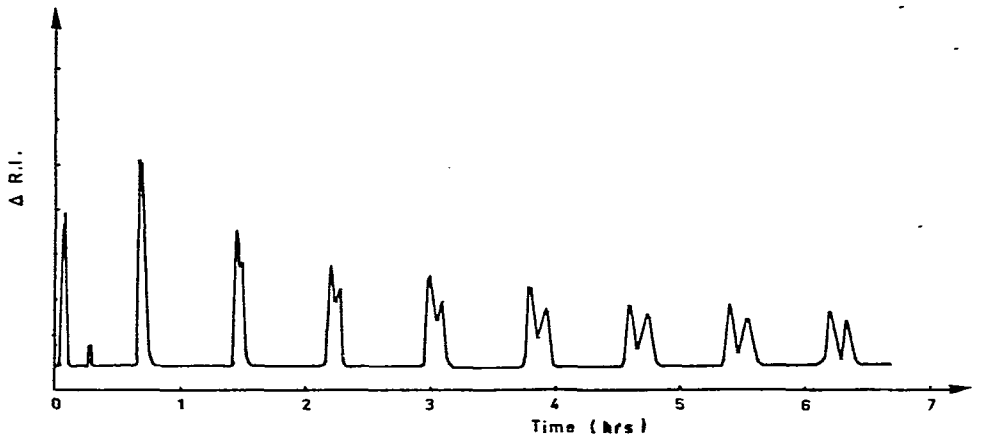

Fig. 2. HPLC of 26-hydroxycholesterol 3,26-diacetate from air-aged cholesterol; separation is achieved after seven recycles. The less polar peak represents the 25*S*-epimer and the more polar one the 25*R*-epimer. Compounds were detected by refractometry.

HPLC of 26-hydroxycholesterol 3,26-diacetate originating from 26-hydroxycholesterol from air-aged cholesterol is shown in Fig. 2; after seven recycles, resolution of the 25*R*- and 25*S*-epimers was achieved, the latter being slightly more abundant (60%) than the former (40%).

DISCUSSION

We have previously found[5] that HPLC in the recycle mode is highly efficient in resolving a 1:1 mixture of (25*R*)- and (25*S*)-26-hydroxycholesterol 3,26-diacetate and provides pure epimers. In the present study, we have established that, even in mixtures containing as little as 3% of either, both epimers could be easily detected and separated.

When applied to 26-hydroxycholesterol 3,26-diacetate obtained by acetylation of 26-hydroxycholesterol from human aortic tissue, HPLC proved unambiguously that this material consisted mainly (90%) of the 25*R*-epimer. Surprisingly, a small amount (10%) of (25*S*)-26-hydroxycholesterol 3,26-diacetate was detected in this material and isolated.

Similar behaviour was observed when 26-hydroxycholesterol 3,26-diacetate derived from kryptogenin of known[4,5] 25*R* configuration was submitted to HPLC. Again, a small amount (3%) of the 25*S*-epimer was detected in this material (hitherto presumed to be optically pure).

The presence of the 25*S*-epimer in 26-hydroxycholesterol from either human aorta or kryptogenin has never to our knowledge been reported. It cannot be ascertained whether it was present in the raw material (a 25*S*-epimer of kryptogenin has recently been described[7]) or whether it arose from epimerization of (25*R*)-26-hydroxycholesterol during chemical manipulations.

We have also tested 26-hydroxycholesterol 3,26-diacetate derived from 26-hydroxycholesterol isolated from air-aged cholesterol[6]. Both 26-hydroperoxy- and 26-hydroxycholesterol have been isolated from this material, but their configuration

at C-25 has not been established because of the lack of adequate methods. Formation of the 26-diol during cholesterol autoxidation has been suggested[6], via homolysis of the peroxy oxygen–oxygen bond, followed by protonation of the intermediate oxy-radical; this should result in equal amounts of the 25*R*- and 25*S*-epimers of 26-hydroxycholesterol. Our results (approximately equal amounts of the epimers) confirm this hypothesis.

The fact that 26-hydroxycholesterol from human aortas is mainly the 25*R*-epimer and not a 1:1 mixture, strengthens the suggestion[2] that the origin of this compound is enzymic and not due to cholesterol autoxidation.

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