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Note

Specific separation of equol from estrogens by thin-layer chromatography

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Equol (4',7-isoflavandiol) was first obtained by Marrian and Haselwood[1] from the urine of pregnant mares, and Klyne and Wright also isolated this compound from the urine of goats[2] and of cows[3]. Macrae *et al.*[4] and Common and Ainsworth[5] presented chromatographic evidence for the presence of equol in the urine and faeces of laying hens[6]. Earlier workers[7] reported the separation of equol from estrogens by thin-layer chromatography (TLC) by making use of *p*-nitrobenzenediazonium tetrafluoroborate in acetic acid–water (1:1) and 2% sulphuric acid in aqueous ethanol[8] as spray reagents. Specific separation of equol is also possible by making use of arsenic trichloride–acetic acid as a spray reagent[9–11].

The present paper describes the specific separation of equol from estrogens by TLC. The procedure involves: (i) separation by TLC of the phenols into (a) estrone, (b) estradiol plus equol and (c) estriol; (ii) ethylation of (b); (iii) subsequent TLC of the ethyl ethers of (b), which affords excellent separation of these two compounds.

A mixture of estrone, estradiol, estriol and equol (3 μg) was chromatographed on silica gel G (E. Merck, Darmstadt, G.F.R.) in benzene–methanol (17:3) in an unsaturated chamber. The two terminal strips of the chromatoplates were sprayed with a 1:1 (w/v) solution of arsenic trichloride in glacial acetic acid while the middle section was protected by a plastic plate, and the plates were heated for 10 min at 110° in order to make the spots visible. The respective R_F values were 0.60, 0.45, 0.24 and 0.45. The blank area corresponding to the estradiol plus equol spot was removed, eluted with ethanol and ethylated[12]. The ethyl ethers were chromatographed in benzene–methanol (17:3), and the spots were detected by spraying with a 1:1 (w/v) solution of arsenic trichloride in glacial acetic acid. Ethylation renders equol less "polar" than estradiol because of the formation of the diethyl ether derivative[2] which travels much further than the 3-ethyl ether of estradiol in this solvent system (R_F = 0.77 and 0.33 respectively).

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