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Note

Preparative chromatography of epimers and anomers of daunomycin derivatives on Sephadex LH-20

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The separation of mixtures of anthracyclinones or anthracycline glycosides prepared by chemical semi-synthesis[1] or microbial products[2] is often difficult, especially when the final stage requires the application of preparative thin-layer chromatography (TLC). In some instances the compounds can be isolated by column chromatography on silica gel and elution with polar solvents. These procedures yielded, *e.g.*, derivatives prepared by acid-catalysed reactions of various alcohols with daunomycinone[3,4], some of which were subjected to glycosidation[5].

Later, it was found that these substances can be separated not only by preparative TLC[6] but also on Sephadex LH-20; the latter method is described in this paper. It was subsequently used to separate in different chromatographic systems the following mixtures of semi-synthetic anthracyclinones: (7*S*,7*R*)-O-(2-hydroxyethyl) daunomycinone (I,II; mixture 1), (7*S*,7*R*)-O-(3-hydroxypropyl)daunomycinone (III,IV; mixture 2), (7*S*,7*R*)-O-(4-hydroxybutyl)daunomycinone (V,VI; mixture 3), (7*S*,7*R*)-O-(6-hydroxyhexyl)daunomycinone (VII,VIII; mixture 4) and (7*S*,7*R*)-7-methoxydaunomycinone (IX,X; mixture 5), in each instance in a mixture with daunomycinone (XV), and mixture of anomers of N-trifluoroacetylated glycosides (XI,XII; mixture 6; and XIII,XIV; mixture 7).

EXPERIMENTAL

The mixtures of anthracyclinones or anthracyclines were obtained according to Jizba and co-workers[3,5] and Přikrylová *et al.*[4]. The structures of the compounds are shown in Fig. 1.

Chloroform, methanol and benzene used as solvents were distilled before use.

Samples were chromatographed on Sephadex LH-20 in an SR-25/1000 column (1000 × 25 mm I.D.). Sephadex LH-20 was pre-swollen in the given chromatographic system for 10 h. All substances under study are coloured and their separation can thus be readily followed. The SR-25/1000 column was fully packed and the sample was then applied in an amount of 100–150 mg in an appropriate solvent system. Chromatography was carried out at 22–25°C.

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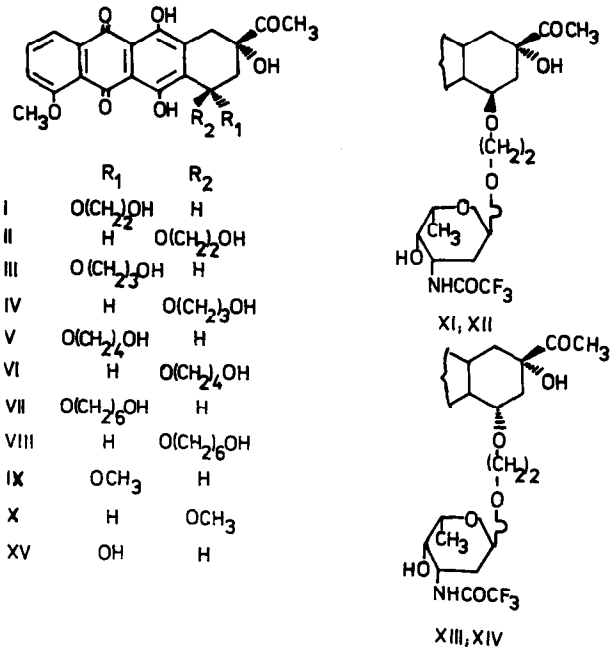

Fig. 1. Structures of the derivatives studied.

RESULTS AND DISCUSSION

The procedure was used for the separation of mixtures of stereoisomers[1–5] and mixtures of semi-synthetic N-trifluoroacetylated anomers of glycosides[6,7]. The results are given in Table I. So far, the separation ability of Sephadex LH-20 has been utilized mostly for separating anthracyclinones from their glycosides[7]. During the reactions[3–5] various by-products are formed that are substituted in position 7, in addition to mono- and bisanhydrodaunomycinone. Under the chromatographic con-

TABLE I

SEPARATION OF MIXTURES OF ANTHRACYCLINES ON SEPHADEX LH-20

Solvent systems: S_1 = chloroform; S_2 = chloroform–benzene–methanol (70:30:0.5); S_3 = chloroform–benzene–methanol (70:30:1.5). Column: SR-25/1000. Flow-rate: 6 ml/min.

Mixture No.	*Elution system*	*Retention volume (ml)**
1	S_1	I, 610; II, 780; XV, 850
1	S_2	I, 685; II, 820; XV, 910
2	S_1	III, 590; IV, 685; XV, 855
3	S_1	V, 485; VI, 570; XV, 840
4	S_1	VII, 460; VIII, 610; XV, 855
5	S_3	IX, 490; X, 575; XV, 860
6	S_1	XI, 440; II, 595; XII, 760
7	S_1	XIII, 490; I, 760; XIV, 875

* For components I–XV, see Fig. 1.

ditions used these compounds are not separated and are obtained in a mixture at an elution volume of 2150–2300 ml with systems S_1, S_2 and S_3. The reaction mixtures 1–7 also contain unreacted original anthracyclinones, which can be separated by this method. In mixtures 6 and 7 formed in the reaction[5] a glycosidic fraction can be separated from an aglycone fraction on Sephadex LH-20 by elution with methanol and the anomers can be separated on the same material with chloroform. During the chromatography of anthracyclinones, the 7*S*-derivative always had a smaller retention time; analogously, in a mixture of glycosides the α-anomer passed through the column at a higher velocity. When the mixtures 1 and 5 were separated with solvent systems S_2 and S_3 the peaks were always less sharp then when chloroform (S_1) was used. However, the volume of the eluted zones reached 60–80 ml only with systems S_2 and S_3, which were better solvents for the chromatographed compounds.

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