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Separation of carotenoid glycosides on magnesium oxide

Secondary and tertiary carotenoid glycosides had not been detected in certain bacteria and blue-green algae until a few years ago (for a review, see LIAAEN JENSEN[1]). These compounds, however, seem to be quite common in several bacterial groups. During our investigations on the carotenoid patterns of myxobacteria, we found several new tertiary carotenoid glycoside esters and carotenoid rhamnosides[2,3]. Some of these bacteria contained up to four closely related compounds that could not be separated by conventional methods. In this communication, a generally applicable isolation procedure for carotenoid glycosides, including a final chromatographic step on magnesium oxide (MgO), is presented.

We have found that MgO (Merck, Darmstadt, G.F.R.) is especially suitable for the separation of peracetylated carotenoid glycosides. Aluminium oxide has been used in the separation of secondary carotenoid glycosides from blue-green algae[4,5], but chromatography on this adsorbent, however, was less useful for our purpose. MgO has been used previously for the separation of carotenoids[6-9], silylated carotenoids[7] and other lipids[10].

The following isolation procedure is used. The crude acetone extract is chromatographed on a silica gel column (length 8 cm, diameter 2 cm) with light petroleum–diethyl ether (1:2, v/v) in order to remove non-glycosidic carotenoids and the bulk of non-polar lipids. Carotenoid glycosides are eluted with diethyl ether and with diethyl ether containing increasing amounts of acetone. Phospholipids remain on the column under these conditions, as has been checked by phospholipid analysis according to the method described previously[11]. The carotenoid glycosides from myxobacteria are generally esterified at one of the glucose hydroxyl groups with various fatty acids. To obtain homogeneous material in the following isolation steps (*e.g.*, for mass spectrometry) saponification of the esters with ethanolic potassium hydroxide is necessary. An important part of the isolation procedure is peracetylation with acetic anhydride in pyridine. By this means the hydrophilic parts of the molecules, *viz.* the sugar moieties, become more hydrophobic. The peracetates exhibit much better chromatographic properties than the native glycosides (see also ref. 4). Further purification is accomplished by a second chromatographic step on a silica gel column. The various carotenoid fractions, however, generally contain more than one component. A final chromatographic step on MgO (thin layer or column) results in a complete separation of the individual glycosides. An unidentified lipid contaminant that occasionally accompanies the carotenoid glycosides is also removed.

The carotenoid glycosides so far identified from myxobacteria, along with their respective R_F values on MgO thin layers, are summarized in Table I. Light petroleum (boiling range 60–70°) –benzene–methanol (40:10:1) is used as the solvent. Column chromatography on MgO with similar solvent mixtures also gives good results for preparative purposes. In this instance, 50 % Kieselguhr (Merck, Darmstadt, G.F R.) is added to the MgO to increase the flow-rate. As can be seen in Table I, additional conjugated carbon–carbon double bonds within the molecules decrease the R_F values, whereas cyclization increases the R_F values. Thus, compounds C, E and H, which move as one zone on silica gel, can be easily separated. The relatively high R_F value

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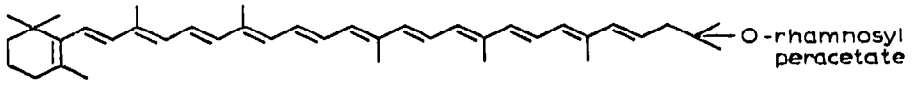
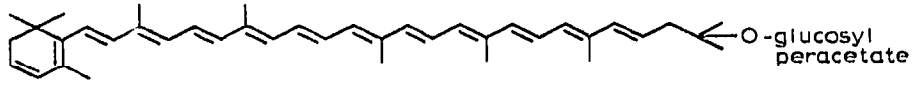
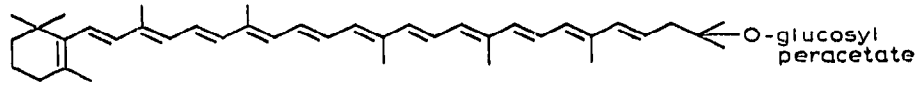
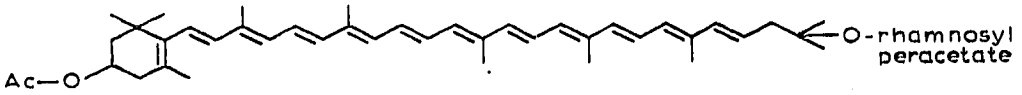
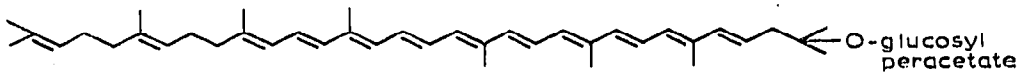
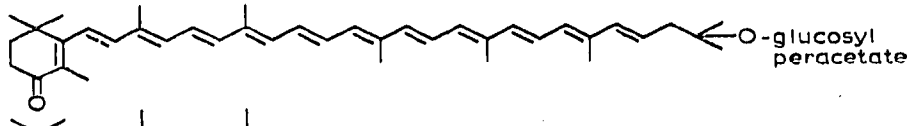
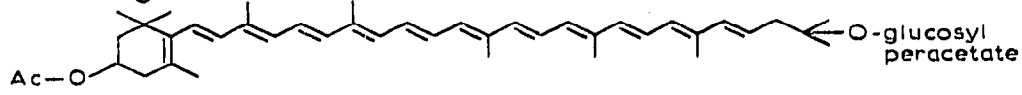
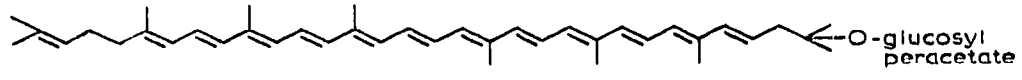
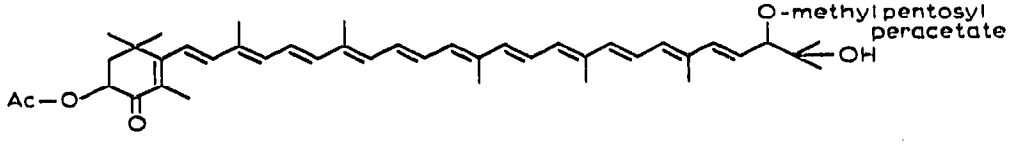
RUCTURAL FORMULAE AND R_F VALUES OF PERACETYLATED CAROTENOID GLYCOSIDES

in-layer chromatography on MgO. Solvent: light petroleum–benzene–methanol (40:10:1).

mpound	*Structural formula*	R_F *value*	*Reference*
	 O-rhamnosyl peracetate	0.68	3
	 O-glucosyl peracetate	0.55	2
	 O-glucosyl peracetate	0.45	3
	 O-rhamnosyl peracetate	0.41	3
	 O-glucosyl peracetate	0.36	–
	 O-glucosyl peracetate	0.23	2
	 O-glucosyl peracetate	0.20	3
	 O-glucosyl peracetate	0.15	–
	 O-methyl pentosyl peracetate, OH	0.54	5

[a] Detailed descriptions of these structures will be published elsewhere.

of compound B on MgO is unexpected. Different types of acetylated hydroxyls (A, C, D and G) or a carbonyl group (F) cause different retardations, and the nature of the glycosidic bond also seems to have a considerable influence on the R_F values. For example, compound I, the peracetate of the secondary glycoside of 4-ketomyxol (isolated from *Plectonema boryanum*, blue-green alga; structure established by FRANCIS *et al.*[5] from *Oscillatoria limosa*) exhibits a relatively high R_F value relative to the tertiary carotenoid glycoside peracetates from myxobacteria.

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