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**Preliminary communication**

# Pyruvic acid derivative of a carrageenan from a marine red alga (*Petrocelis* species)

VINCENT DiNINNO, ESTHER L McCANDLESS, and RUSSELL A BELL

*Departments of Biology and Chemistry, McMaster University, Hamilton, Ontario L8S 4K1 (Canada)*

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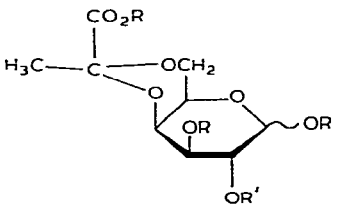
In the course of investigating carrageenans from various red algal species, we have observed a unique, highly sulphated carrageenan in which the repeating disaccharide units are almost completely substituted with pyruvic acid The carrageenan from *Petrocelis middendorfii* (*P franciscana*)[1]* collected at Mission Pt , Monterey Co , CA, was extracted as described by McCandless *et al.*[2] This carrageenan has recently been reported[3] to contain 32% sulphate groups and essentially no 3,6-anhydro-D-galactose residues Upon treatment with sodium borohydride, the content of 3,6-anhydro-D-galactose increased only to 5% The significance of this to the sulphation pattern and immunochemical reactivity to an anti-λ-carrageenan has been discussed earlier[3]

In this carrageenan preparation, pyruvic acid was detected by the enzymic procedure of Duckworth and Yaphe[4] This result was supported by the p m r spectrum of the methanolysate (obtained as described below) in dimethyl sulphoxide-$d_6$ solution, which showed the presence of a single methyl peak at δ 1 44, in agreement with the assignment given by Morris *et al.*[5] for pyruvic acid in xanthan The pyruvic acid content was estimated to be 6 5% by p m r of the native polysaccharide in deuterium oxide, at 75° and 90 MHz, with 1 μmol of sodium acetate/2 mg of polysaccharide as the internal standard

The pyruvic acetal, 4,6-*O*-(1-carboxyethylidene)-D-galactose residue (**1**), was first reported in agar by Hirase[6–8], and was subsequently reported to occur in low con-




1 R = R = H
2 R = Me , R' = $F_3CO$

*P franciscana* has been reduced to a synonym of *P middendorfii*[1]

centration in agars prepared from a number of agarophytes[9,10], and in microbial polysaccharides[11]. The same acetal residue has also been demonstrated in a carrageenan extracted from the marine red alga *Gigartina tenella*, and in a polysaccharide from *Grateloupia elliptica*. The carrageenan from *G tenella* contained 1 5% of pyruvic acid, a proportion equivalent to 1 pyruvic acid group per 20 sugar residues[12].

To determine whether the pyruvic acid group in the *Petrocelis* carrageenan occurred as the acetal **1**, the carrageenan (1 mg) was submitted to methanolysis with M hydrogen chloride in anhydrous methanol (1 mL) for 20 h at 80°. The methanolic hydrogen chloride was evaporated, and the trifluoroacetates of the methyl glycosides were prepared by treating the methanolysate with an excess (0 2 mL) of trifluoroacetic anhydride for 1 h at 80° The *O*-trifluoroacetyl derivatives were analyzed by g l c on a glass column (180 X 0 7 cm o d ) containing 3% OV-210 Chromosorb W (80–100 mesh) The column temperature was 150°, and that of the flame ionization detector 350°, the nitrogen carrier gas-flow was 40 mL/min. The results of the g l c. (Fig 1) indicate the presence of methyl *O*-trifluoroacetylgalactosides (retention times 3 94, 4 42, and 6 05, $R_{myo\text{-inositol}}$ 1 39, 1.56 and 2 14, respectively) and substituted trifluoroacetylgalactosides (retention times 9 51 and 13 53, $R_{myo\text{-inositol}}$ 3 36 and 4 78, respectively) The difference between

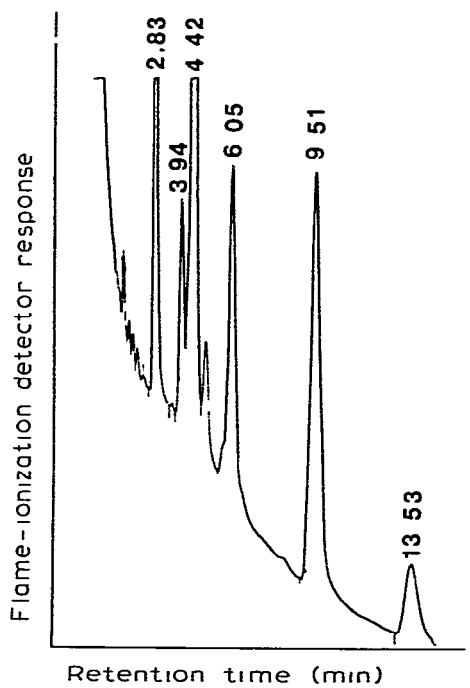
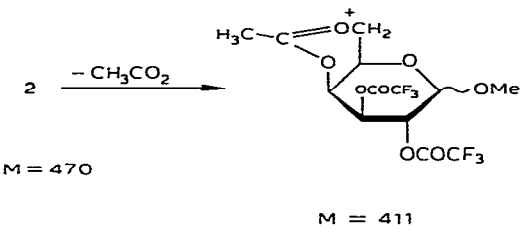



Fig 1 G.l c pattern of the *O*-trifluoroacetyl derivatives of the methyl glycosides obtained by methanolysis of π-carrageenan The retention time of 2 83 min is that of the internal standard *myo*-inositol The retention times of 3 92, 4 42, and 6 05 min correspond to methyl *O*-trifluoroacetyl-D-galactosides, and the retention times of 9.51 and 13 53 min correspond to **2**

the two sets of retention times agrees closely with the difference observed by Hirase and Watanabe[12] for the trimethylsilyl derivatives of the methyl glycosides of D-galactose and **1**. In this sample, however, the methyl *O*-trifluoroacetylgalactosides and methyl 4,6-*O*-(1-carboxyethylidene)-*O*-trifluoroacetyl-D-galactosides occurred in a molar ratio of 2.1, which represents 1 D-galactose pyruvate per 1 5 disaccharide units

To obtain definitive proof of the presence of the acetal **1**, the *O*-trifluoroacetyl derivatives of the methanolysates were separated by g l c on a glass column (180 × 0 4 cm o d ) containing 3% of OV-17 Chromosorb W (80—100 mesh) at a column temperature of 150°, and fed directly into a mass spectrograph The m s was recorded at an ion source temperature of 200°, ionizing potential of 70 eV, and accelerating potential of 3 kV The resulting fragmentation pattern of the compound with retention time 9 51 (Fig 1) was consistent with that expected for **2** Although the parent ionic species was not obtained, the major ionic species (100% I/B) and its derivation from the parent species are shown in Scheme 1




Scheme 1

We have found the same pyruvic acid acetal, in lower concentrations, only in carrageenans from several other *Petrocelis* species and from certain related *Gigartina* species Recent evidence suggests that *Petrocelis middendorfii* (*P franciscana*) represents the sporophyte generation of *Gigartina papillata*[1] Thus, the occurrence of pyruvic acid bound to carrageenan may have taxonomic significance

Unlike agar, in which the pyruvic acid acetal groups occur in a fraction having little sulphate ester or being remote from sulphated regions of the molecules[13,14], the polysaccharide from *Petrocelis middendorfii* (*P franciscana*) is composed entirely of 2-sulphate residues, as shown by i r , periodate oxidation, and alkaline borohydride reduction[3] The pyruvate group must occur in association with these sulphated sugar residues As the backbone of carrageenan molecules consists of α-(1→3)- and β-(1→4)-linked D-galactopyranosyl residues, we propose that the carrageenan from *Petrocelis middendorfii* (*P franciscana*) contains a repeating structure consisting primarily of alternating (1→3)-linked 4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranosyl 2-sulphate and (1→4)-linked α-D-galactopyranosyl 2-sulphate residues The major component in the *Petrocelis* species and a minor component in Hirase's polysaccharides[12] thus represent a new carrageenan type, which we propose to call π-carrageenan A small proportion of (1→3)-linked β-D-galactopyranosyl 2-

sulphate and (1→4)-linked α-D-galactopyranosyl 2,6-disulphate residues are present in the *Petrocelis* carrageenan It is impossible to state whether these are dispersed among the π-carrageenan units, or whether they represent a contaminating λ-carrageenan

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