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SHORT COMMUNICATION

AN EXTRACTION PROCEDURE FOR PLANTS: EXTRACTS FROM THE RED ALGA *RHODOMELA LARIX*

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Abstract—An extraction procedure using an homogeneous mixture of chloroform–methanol–water has proved useful in isolating natural products from algae. The extracted components of the red alga *Rhodomela larix* were readily separated into water-soluble and insoluble fractions, the former consisting mainly of sodium 2-*O*-(α-D-mannopyranosyl)-D-glycerate and D-mannitol together with trace amounts of other constituents, all of which were biologically inactive.

THE HOMOGENEOUS solvent mixture of chloroform–methanol–water, initially used for the isolation of lipids from fish,[1] has furnished extracts from algae suitable for microbiological examination. The procedure, which can be used in isolating natural products from plants, is illustrated in this communication with the extraction of the red alga *Rhodomela larix*. It has been reported[2] that an 80 per cent aqueous methanol extract of this alga has been separated into biologically active water-soluble and insoluble fractions. The effective agents of the latter fraction were concluded to be bromophenolic compounds and recently two have been identified as 2,3-dibromo-4,5-dihydroxybenzaldehyde and 2,3-dibromo-4,5-dihydroxybenzyl methyl ether.[3] The observation that the water-soluble fraction inhibited the growth of *Escherichia coli* prompted an interest in the low molecular weight, water-soluble components of this alga.

The seaweed was exhaustively extracted in a Waring Blendor with the solvent mixture chloroform–methanol–water (v/v, 1:2:0·5) and the resultant combined and filtered extracts made biphasic by the subsequent addition of chloroform and water (v/v, 1:1). The chloroform layer was separated and concentrated to a tar (A) in 0·7 per cent yield (fresh weight basis). The remaining aqueous methanol layer was concentrated to a residue 0·6 per cent yield, which as an aqueous solution was passed through a column of cation then anion exchange resins to afford 0·05 per cent yield of neutral components (B).

Separation of fraction (B) on Whatman 3MM filter sheets gave D-mannitol as the major constituent, which was identified by comparison with an authentic specimen, m.p., mixed m.p. 168°, and by the proton magnetic resonance spectrum and gas chromatographic mobility of the derived hexaacetate. Minor amounts of other constituents present in fraction (B) have not, as yet, been identified. Elution of the anion exchange resin with formic acid afforded

[1] E. G. BLIGH and W. J. DYER, *Can. J. Biochem. Physiol.* **37**, 911 (1959).
[2] K. SAITO and M. SAMESHIMA, *Nippon Nogei-Kagaku Kaishi* **29**, 427 (1955).
[3] N. KATSUI, Y. SUZUKI, S. KITAMURA and T. IRIE, *Tetrahedron* **23**, 1185 (1967).

crystalline sodium 2-*O*-(α-D-mannopyranosyl)-D-glycerate 1 which was identified by comparison with an authentic sample. The corresponding derivative methyl 3-*O*-acetyl-2-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyl)-D-glycerate has been subjected to mass and proton magnetic resonance spectrometry to affirm the assigned structure.[4]

The remaining alga from the above procedure was further extracted for 7 days with 80 per cent aqueous methanol in a Soxhlet extractor to yield 0·01 per cent of extractives. A comparison of the yields demonstrated the efficiency of the former extraction procedure which, unlike Soxhlet extraction, permits the isolation of thermally unstable components, and offers a ready separation of constituents into water-soluble and insoluble fractions suitable for further separation.

The sodium salt 1 and fraction (B) exhibited no antibacterial activity when tested against Gram positive and negative bacteria. The inhibition of *Sarcina lutea* and *Pseudomonas perfectomarinus* by the chloroform-soluble fraction (A) is probably indicative of the presence of bromophenolic compounds previously mentioned.

EXPERIMENTAL

General

Paper chromatography was performed on Whatman No. 1 paper using the following solvent systems (v/v): (*a*) EtAc–HAc–formic acid–H_2O (18:3:1:4), (*b*) EtAc–pyridine–water (10:4:3), (*c*) *n*-BuOH–EtOH–H_2O (4:1:5, upper layer). Detection of the compounds on paper chromatograms was accomplished with alkaline $AgNO_3$.[5] Proton magnetic resonance (PMR) spectra were obtained with a Varian HA 100 spectrometer; τ values are relative to tetramethylsilane as an internal standard.

Isolation

Rhodomela larix (800 g, fresh wt.), freed from extraneous marine flora and fauna was successively extracted in a Waring Blendor (1 gal capacity) with the solvent mixture, $CHCl_3$–MeOH–H_2O v/v (1:2:0·5), until no further residue was obtained on evaporation of a portion of the filtered solvent extract (10 l.). The addition of $CHCl_3$ (1 l.) and H_2O (1 l.) to the combined filtered extracts afforded a $CHCl_3$ layer which was separated and concentrated to a tar (A) (6 g).

The remaining aqueous MeOH layer was evaporated to a residue (5 g) which as an aqueous solution was passed through a column of Rexyn resin 101 (H) then a column of Duolite resin A4 (OH) to give a mixture of neutral components (B) (400 mg) which was shown by paper chromatography to consist of a compound with $R_{glucose}$ 1·3, 0·94, and 1·04 in solvents (*a*), (*b*) and (*c*) respectively, with minor amounts of components having $R_{glucose}$ 1·0, 0·68 and 0·57 in solvent (*a*).

A portion of the mixture (B) (200 mg) was separated on Whatman 3MM filter sheets by elution with solvent (*a*) to give the major component D-mannitol (50 mg), m.p., mixed m.p. 168°, whose derived hexaacetate, m.p., mixed m.p. 126°, gave a PMR spectrum in $CDCl_3$ exhibiting an unsymmetrical doublet at τ 4·54 for H-3 and H-4, a complex multiplet at τ 4·91 for H-2 and H-5, two quartets for the methylene protons H-1_a (≡H-6_a) and H-1_b (≡H-6_b) at τ 5·76 and 5·95 respectively, with $J_{1a-2} = 2·9$ Hz (≡J_{6a-5}), $J_{1b-2} = 5·0$ Hz (≡J_{6b-5}) and $J_{1a-1b} = 12·5$ Hz (≡J_{6a-6b}) together with three signals at τ 7·92, 7·94 and 7·96 corresponding to six acetoxyl groups. The gas chromatographic mobility of the derived hexaacetate was identical to that of an authentic specimen.[6]

Elution of the Duolite resin A4 with 0·2 N formic acid and repeated evaporation with water to 50 ml gave an acid solution which was neutralized with $NaHCO_3$ and concentrated to dryness. Extraction of the residue with hot 90% aqueous MeOH yielded a combined extract which was filtered and concentrated to a product subsequently recrystallized several times from aqueous MeOH to afford a crystalline salt 1 (300 mg), m.p. 265°, $[\alpha]_D^{25} + 106°$ (*c.* 1·4 in water), $R_{glucose}$ 1·05, 0·14 and 0·17 in solvents (*a*), (*b*) and (*c*) respectively. Hydrolysis of a portion of 1 with N HCl at 100° for 1 hr furnished a hydrolysate, shown by paper chromatography in solvents (*a*), (*b*) and (*c*) to contain mannose and glyceric acid. The chromatographic mobility of 1 was found to be identical to a specimen of sodium 2-*O*-(α-D-mannopyranosyl)-D-glycerate.

The remaining alga from the above extraction procedure was extracted for 7 days with 80% aqueous MeOH in a Soxhlet extractor and the resulting solution filtered and evaporated to a residue (90 mg).

[4] J. N. C. WHYTE, *Can. J. Chem.*, **47**, 4083 (1969).
[5] W. E. TREVELYAN, D. P. PROCTER and J. S. HARRISON, *Nature* **166**, 444 (1950).
[6] P. ALBERSHEIM, D. J. NEVINS, P. D. ENGLISH and A. KARR, *Carbohyd. Res.* **5**, 340 (1967).

Microbiological Testing

Samples of fraction (A) (10% in acetone), fraction (B) and the sodium salt 1 (10% in water) were added to 12·7 mm diam. filter discs, dried, and placed on a nutrient aga (Difco) subsequently innoculated with the Gram positive bacteria, *Micrococcus aquivivus*, *Sarcina lutea* and unclassified *Streptococcus* and Gram negative bacteria, *Escherichia coli* and *Pseudomonas perfectomarinus*. No growth-inhibiting activity was observed for fraction (B) and the sodium salt 1, but fraction (A) inhibited growth of *S. lutea* and *P. perfectomarinus*.

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