Planta (Berl.) 115, 83—86 (1973)

Short Communication

Simple Brominated Phenols in the Bluegreen Alga *Calothrix brevissima* West

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Received July 31 / September 12, 1973

Summary. Through the use of gas chromatography-mass spectrometry techniques two simple brominated phenols, 2,3-dibromo-4,5-dihydroxybenzyl alcohol (lanosol) and 3,5-dibromo-*p*-hydroxybenzyl alcohol, were identified in an axenically cultured bluegreen alga, *Calothrix brevissima*. The alga was grown in a mineral medium supplemented with 1% glucose and 0.075% NaBr for 3 weeks. The culture filtrate contained two tribrominated phenols, 2,3,5-tribromo-*p*-hydroxybenzyl alcohol and 2,3,5-tribromo-*p*-hydroxybenzaldehyde, and traces of lanosol.

The occurrence and distribution of simple brominated phenols in the *Rhodomelaceae* have been investigated chromatographically (Péguy, 1964). In describing 3,5-dibromo-*p*-hydroxybenzyl alcohol as a natural constituent of *Odontalia dentata* and *Rhodomela confervoides*, Craigie and Gruenig (1967) have briefly summarized previous reported findings of bromophenols in other Rhodomelacean species. Further, in a survey (Pedersén *et al.*, 1974) of 23 Rhodophycean species, these and related compounds have been reported in both Rhodomelaceae and other red algae. Lanosol appeared to be the most abundant and widely distributed bromophenol; it was identified in eight species. To our knowledge no information on the occurrence of these compounds in the procaryotic bluegreen algae have been published. We now describe the isolation and identification of lanosol from the bluegreen alga *Calothrix brevissima* West.

The alga was obtained in the axenic state from Dr. T. Ichimura, Institute of Applied Microbiology, University of Tokyo. It was grown in the mineral medium GO (Holm-Hansen, 1964) supplemented with 1% glucose and 0.075% NaBr, at 30° and 3000 lux continuous light (fluorescent lamps, Philips TL/33) in a controlled growth room for 3 weeks.

Samples of 2 g dry weight of the alga were extracted with 80% aqueous acetone for 30 min in a ultrasonic bath (Branson Instr. Co., Stamford, Conn., USA) under cooling to 30°. Following evaporation of the acetone under reduced pressure, the

aqueous phase was filtered through prewashed Hypoflow celite. Removal of esterified sulphates was achieved by acidification to pH 2 with 2 N HCl and simultaneous heating for 10 min. The resulting solution was cooled and extracted 3 times with 30-ml aliquots of ethylacetate; the fractions thus obtained were pooled and the solvent was evaporated. The residue was redissolved in a small volume of ethylacetate, transferred to a microsilylation vessel, and concentrated to dryness under nitrogen and further over phosphorus pentoxide to ensure the absence of any traces of water. BSTFA (N,O-bis-trimethylsilyl-trifluoroacetamide) with 1% TMCS (trimethylchlorosilane, Pierce Chemical Co. No. 38831) as catalyst, and acetonitrile were added at 50 µl each, the entire mixture heated at 60° for 15 min and subjected to gas liquid chromatography (GLC).

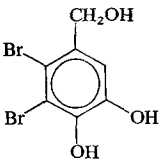
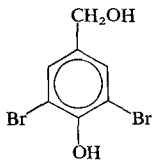
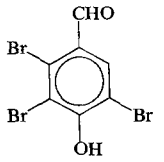
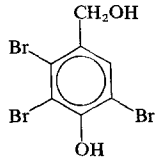
The culture filtrate was concentrated to *ca.* 200 ml, acidified to pH 2, heated to 70° for 10 min, extracted 3 times with ethylacetate, and then subjected to the same extraction and silylation procedure as above. Samples of 2 µl were employed for the GLC and mass-spectral (MS) analyses.

Analyses were carried out on a Varian Aerograph gas chromatograph model 1740 and on a 1.22 m, 4 mm i.d., silanized glass column packed with 3% (w/w) SE 30 on 80/100 mesh acid-washed, DMCS-treated Chomosorb W. Retention times were determined over a temperature programme of 100–250° at 6° min^{-1}. Injector and detector temperatures were 220° and 240°, respectively. The mass spectra were determined with a CH_7 Varian Mass spectrometer coupled to the gas chromatograph. Helium was employed as the carrier gas at the flow rate of 25 ml min^{-1}.

MS analysis showed the presence of 2,3-dibromo-4,5-dihydroxybenzyl alcohol (silylated molecular weight 512) and 3,5-dibromo-*p*-hydroxybenzyl alcohol (silylated MW 424) in *Calothrix brevissima*. The culture medium contained a tribrominated aldehyde (2,3,5-tribromo-*p*-hydroxybenzaldehyde) and alcohol (2,3,5-tribromo-*p*-hydroxybenzyl alcohol) with silylated MWs of 428 and 502, respectively. Traces of lanosol were also encounted in the culture filtrate. The retenion times observed for lanosol and the dibromo and tribromo compounds are given in Table 1, together with the proposed structures. Lanosol appeared at 233°, whereas the dibrominated hydroxybenzyl alcohol appeared at 209°. In the case of the tribrominated compounds present in the culture filtrate, the aldehyde was detected at 206° whereas the alcohol was observed at 233°. In the case of these two compounds the proposed positions of the bromine atoms are based on the position of those in the 2,3- and 3,5-dibromophenols, respectively. The significant peaks of the TMS derivatives of these tribromophenols were at MS: (M^+) 502, 423, 335, 177, 135 and 73 and MS (M^+) 428, 414, 335, 257, 147 and 73.

Retention times and mass spectra of the bromophenols from *Calothrix brevissima* and its culture medium were in entire agreement with the same parameters from the same compounds isolated and identified in red alga (Pedersén *et al.*, 1974). The dibromo-monohydroxy- and dibromo-dihydroxyalcohols are known to occur in the esterified sulphate state (Hodgkin *et al.*, 1966; Glombitza and Stoffelen, 1972). A decrease in antibiotic activity has been ascribed to the presence of sulphate esters

Table 1. Retention times and proposed structures of the brominated phenols

Compound	Silylated (MW)	Source	Retention time with programme 100° at 6° min^{-1} (min)	Proposed structure
2,3-dibromo-4,5-dihydroxy-benzyl alcohol (lanosol)	512	*Calothrix brevissima*	22.2	
3,5-dibromo-*p*-hydroxybenzyl alcohol	424	*Calothrix brevissima*	17.6	
2,3,5-tribromo-*p*-hydroxybenz aldehyde	428	Culture filtrate of *C. brevissima*	17.6	
2,3,5-tribromo-*p*-hydroxybenzyl alcohol	502	Culture filtrate of *C. brevissima*	22.2	

(McLachlan and Craigie, 1966), desulphated lanosol being found toxic to unicellular marine algae. The reported presence of lanosol in the water of the *Polysiphonia Brodiaei* zone (Pedersén *et al.*, 1974) indicates the exudation from the alga of this metabolite into the immediate surroundings. Fries (1973) has observed that lanosol stimulates the growth of some red algae in axenic culture. The findings of lanosol and the tribromophenols in the culture filtrate of *Calothrix brevissima* is interesting with respect to the growth of the alga in its natural habitat, particularly since bluegreen algae have been reported to contain antibacterial and antifungal factors (Welch, 1962). Crude filtrates obtained after growth of bluegreen algae are also reported to stimulate the growth of unicellular algae and plant and animal tissue cultures (Lefévre, 1964).

The authors thank Dr. L. Fries, Institute of Physiological Botany, University of Uppsala, for her advice and encouragement, and Professors O. Theander and L. Lundgren, Department of Chemistry, Agricultural College, Uppsala, for valuable help with the mass spectrometer. One of us (E.J.S.) acknowledges the award of a UNESCO fellowship. This investigation was supported by a Swedish Natural Research Council grant to Dr. L. Fries.

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