

THE OCCURRENCE OF PROSTAGLANDINS PGE₂ AND PGF_{2α} IN A PLANT - THE RED ALGA
GRACILARIA LICHENOIDES.

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Summary. Prostaglandins PGE₂ and PGF_{2α} were isolated from the aqueous extract of the red alga Gracilaria lichenoides, after an investigation of the extract's anti-hypertensive properties.

All naturally occurring prostaglandins have been found in animals, particularly mammals^{1,2}. Here we present the first reported occurrence of prostaglandins in a plant. Prostaglandins PGE₂ (1) and PGF_{2α} (2) have been isolated from the red alga Gracilaria lichenoides. Another marine organism, the soft coral Plexaura homomalla, has been studied extensively³ and several prostaglandins, including PGE₂, isolated from this primitive animal. G. lichenoides (8.3 kg wet weight, 945 g dry weight) was collected from West Head, Victoria, Australia, frozen, stored at -20° then cryogenically ground in liquid nitrogen. Extraction of the ground organism twice with water (15 l, 0°; 10 l, 10°) followed by lyophilisation of the combined extracts afforded a powder A (456.5 g, 48% of the dry organism). A displayed potent anti-hypertensive activity when given intravenously to pentobarbitone-anaesthetised, hypertensive rats. Isolation of the anti-hypertensive agent from G. lichenoides was achieved by a sequence of chromatographic separations guided at each step by the hypertensive rat bioassay.

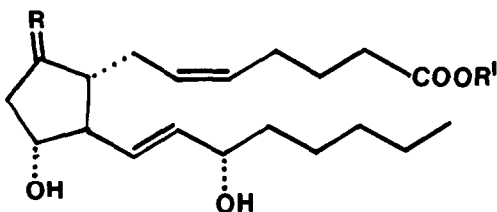
Aqueous extract A (260 g) was adsorbed onto Amberlite XAD-2 in water and the active fraction B (1.99 g, 0.37%) was eluted with methanol. Fraction B (1.95 g) was chromatographed on Sephadex G-25 in water and the anti-hypertensive activity of the eluate was confined to a fraction C (780 mg, 0.15%) eluted at V_R/V_M 2.00 - 2.73. Preparative HPLC of C (750 mg) on octadecyl silica with a methanol-water stepwise gradient resulted in the elution of an active fraction D (200 mg, 0.04%) in water: methanol (6:4). Further HPLC of D (200 mg), run isocratically in water: methanol (6:4), afforded the anti-hypertensive agent (1) (27 mg, 0.006%) and a chromatographically similar, inactive constituent (2) (43 mg, 0.008%).

After the spectral, optical rotation and pharmacological data on (1) and (2) were examined it was deduced that they were slightly impure PGE₂ and PGF_{2α} respectively. To facilitate complete characterisation, and the attainment of optical purity, (1) and (2) were esterified

with diazomethane and the esters, (3) and (4) respectively, purified by preparative TLC (benzene: dioxan, 5:4)⁴. It was proved unequivocally that (3) and (4) were the methyl esters of PGE₂ and PGF_{2α} by comparing them with the ¹³C NMR⁵, ¹H NMR⁴, TLC⁴, MS⁶⁻⁹ (underivatized and TMS derivatives) and optical rotation data¹⁰ from the literature and with authentic samples¹⁰.

A semi-quantitative analysis of the amount of PGE₂ present in the alga was accomplished, in the absence of deuterated derivatives for selected ion mass spectrometry, by extracting an acidified (pH 2.7), aqueous extract with ethyl acetate¹¹. It was estimated from a correlation of the dose-response curves of the anti-hypertensive activity of PGE₂ and the ethyl acetate extract, that PGE₂ constitutes 0.05-0.07% (dry weight) of *G. lichenoides*. By relating the amount of PGF_{2α} to PGE₂ it was calculated that PGF_{2α} constitutes 0.07-0.10% (dry weight) of the organism. A macroscopic investigation of the alga revealed that it was free of symbionts and contaminants.

The fact that PGE₂ and PGF_{2α} occur together, and appear to be the only prostaglandins present, invites speculation that they are derived from arachidonic acid, the mammalian biosynthetic precursor² to PGE₂ and PGF_{2α}. It is noteworthy that the lipoxygenase-2 enzyme from the soybean plant can transform arachidonic acid into PGF_{2α}¹². Preliminary analyses of an extract of *Gracilaria confervoides* indicate that prostaglandins may be present.



- (1) R = O; R' = H
 (2) R = αOH, βH; R' = H
 (3) R = O; R' = CH₃
 (4) R = αOH, βH; R' = CH₃

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References and Notes

1. S. Bergstrom, *Science*, **157**, 382 (1967).
2. P.W. Ramwell, "The Prostaglandins", Vol. 1, Plenum Press, New York, 1973.
3. F.M. Bayer and A.J. Weinheimer, "Studies in Tropical Oceanography" No. 12, University of Miami Press, Florida, U.S.A., 1974.
4. P.W. Ramwell, J.E. Shaw, G.B. Clarke, M.F. Grostic, D.G. Kaiser and J.E. Pike, *Progr. Chem. Fats and Other Lipids*, **9**, 231-273 (1968).
5. G.F. Cooper and J. Fried, *Proc. Nat. Acad. Sci. U.S.A.*, **70**, 1579-1584 (1973).
6. G. Horvath and G. Ambrus, *Biomed. Mass Spec.*, **5**, 544-550 (1978).
7. G. Horvath, *Biomed. Mass Spec.*, **3**, 127-136 (1976).
8. G. Horvath, *Biomed. Mass Spec.*, **3**, 4-13 (1976).
9. E.O. Oswald, D. Parks, T. Eling and B.J. Corbett, *J. Chromat.*, **93**, 47-62 (1974).
10. We thank G.L. Bundy for the optical rotation data and F. Kienzle for gifts of PGE₂ and PGF_{2α}.
11. Analogous to procedure used by W.P. Schneider, G.L. Bundy, F.H. Lincoln, E.G. Daniels and J.E. Pike, *J. Amer. Chem. Soc.*, **99**, 1222-1232 (1977).
12. G.S. Bild, S.G. Bhat, C.S. Ramadoss and B. Axelrod, *J. Biol. Chem.*, **253**, 21-23 (1978).