Tetrahedron Letters No. 20, pp 1737 - 1738, 1976. Pergamon Press. Printed in Great Britain.

THE STRUCTURE OF PROSTRATIN : A TOXIC TETRACYCLIC DITERPENE ESTER FROM PIMELEA PROSTRATA

A.R Cashmore*+ and R.N. Seelye++
Chemistry Department, University of Auckland, New Zealand
B.F. Cain
Cancer Research Laboratory, Mt Albert, Auckland, New Zealand
H. Mack, R. Schmidt and E. Hecker
Institut für Biochemie, Deutsches Krebsforschungszentrum, Heidelberg, Germany

(Received in UK 16 March 1976, accepted for publication 5 April 1976)

Pimelea prostrata, an endemic New Zealand shrub, has been responsible for poisoning of livestock in New Zealand[1,2]. Similarly, in Australia, Pimelea species are responsible for St. George disease in cattle[3]. Plant extracts are also of interest owing to their anti-tumor properties[4,5]. Here we report the characterisation of the major toxic component from Pimelea prostrata.

Benzene extraction of the dried plant, followed by partition chromatography on propylene glycol impregnated cellulose, isolated prostratin (1), $C_{22}H_{30}O_6$, m p. 217-218°; ν max ($CHCl_3$) 1705 and 1725 cm^{-1}, λ max (EtOH) 201, 235 and shoulder at 260 (ε 11 100, 5100 and 3400) nm, $(\alpha)_D$ (C, 0.13 in MeOH) + 64°. Prostratin contains a readily esterified hydroxyl group, indicated by the formation of the acetate (2), m.p. 120-125°; m/e 432.2149 ($C_{24}H_{32}O_7$ reqs. 432.2148), δ ($CDCl_3$) 4.48 (H-20, s). The acetate (2) shows strong hydroxyl absorption in the ν spectrum and this is not removed by refluxing (2) in acetic anhydride and pyridine. Oxidation with solid MnO_2 characterised an allylic primary hydroxyl group by the formation of the $\alpha\beta$-unsaturated aldehyde (3), m.p. 230-232°, m/e 388.1888 ($C_{22}H_{28}O_6$ reqs. 388.1886), λ max (EtOH) 240 (ε 15400) nm, δ($CDCl_3$) 9.28 (H-20, s).

Both the acetylation product (2), and the oxidation product (3), showed two deuterium exchangeable protons in their nmr spectra, indicating that prostratin is a trihydroxy compound. CrO_3 oxidation of prostratin yielded (3) confirming the tertiary nature of two of the hydroxyls. The remaining oxygen atom of prostratin forms part of an $\alpha\beta$-unsaturated ketone and both the ν spectrum and the low field resonance of proton 1 in the nmr spectrum (Figure 1) indicates a pent-1-en-3-one system. The evidence presented so far, including the observed coupling in the nmr spectra allows the construction of the following partial formula for prostratin

CO - C (CH_3) = CH - CH, CH - CH - CH = C - CH_2 - OH, CH_3 - CH, CH_2, 2 CH_3, 2 CH, CH_3-CO-O, C_5H_2.

+ Present address Applied Biochemistry Division, DSIR, Palmerston North, New Zealand
++ Present address: Pathology Department, School of Medicine, University of Auckland, New Zealand.

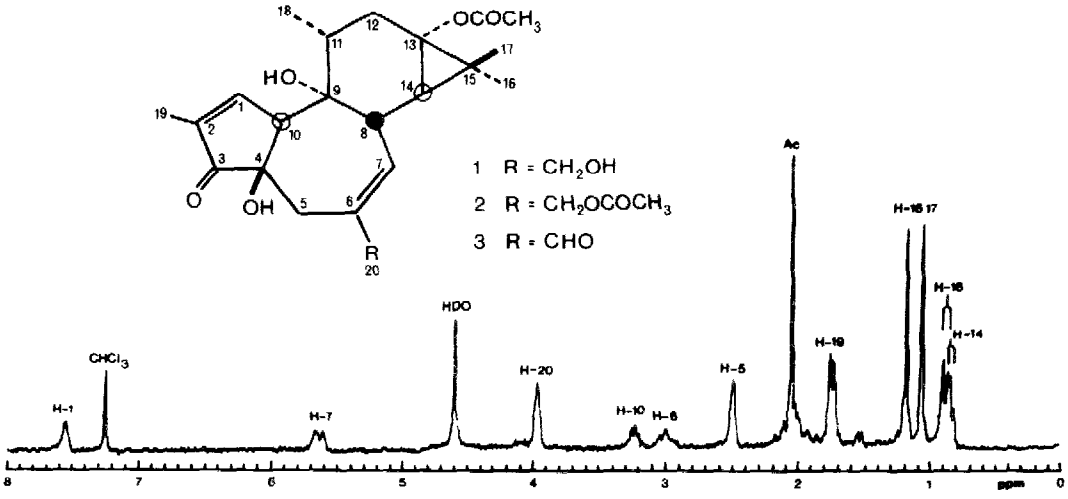

Figure 1. 100 MHz nmr spectrum of prostratin (1) in $CDCl_3$ and D_2O.

With the publication of the structures of the co-carcinogenic phorbol esters from croton oil[6] it was apparent to us that prostratin was most likely represented by structure (1). We have confirmed this formulation by a detailed comparison of the physical properties of the acetate of prostratin (2) and 12-desoxy-phorbol-13,20-diacetate[7]. By tlc, mass and nmr spectra these two compounds are shown to be identical. Furthermore, the structure of prostratin has been independently determined by X-ray crystallography[8].

Prostratin is highly toxic; an intraperitoneal injection of 56 µg being fatal within 2h for an 18 g mouse. Prostratin does not possess the co-carcinogenic activity associated with phorbol esters.

Acknowledgements: We thank J.S Shannon, C.S.I.R.O., Sydney, Australia and R. Hodges, Massey University, Palmerston North, New Zealand for measurement of mass spectra.

References

1. W.M. Webster, N Z J. Agr., 33, 102 (1926)
2. B.C. Aston, N Z.J. Agr., 49, 150 (1934)
3. H.B. Roberts, T.J. McClure, E. Ritchie, W.C Taylor and P.W. Freeman, Aust. Vet. J. 51, 325 (1975)
4. B.F. Cain, unpublished.
5. H.T.C. Howard and M.E.H. Howden, Cancer Chemotherapy Reports, 59, 585 (1975)
6. E. Hecker, H. Bartsch, H. Bresch, M. Gschwendt, E. Harle, G. Kreibich, H. Kubinyi, H.U Schairer, Ch. v Szczepanski and H.W. Thielmann, Tetrahedron Lett., 33, 3165 (1967); see also . E. Hecker and R. Schmidt, Prog. Chem. Org. Natur. Products, 31, 377 (1974).
7. M. Gschwendt and E. Hecker, Tetrahedron Lett , 40, 3509 (1969)
8. I.R.N. McCormick, P.E. Nixon and T.N Waters, Tetrahedron Lett., 20, 1735 (1976)