The Chemistry of Prasinons A and B. Identification of the Nitrogen-containing Moiety

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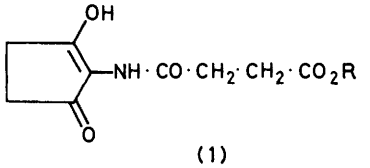
Hydrogenolysis of prasinon B, an insecticidal metabolite of *Streptomyces prasinus*, gave *N*-2-(1,3-dioxocyclopentyl)-succinamic acid, the methyl ester of which (dihydroflavensomycinic acid) is a known degradation product of the antibiotic flavensomycin. The relationship between flavensomycin and the prasinons is discussed.

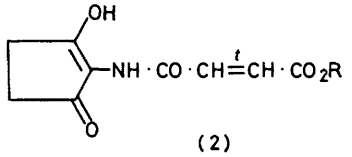
PRASINONS A and B are insecticidal metabolic products of *Streptomyces prasinus*.[1] Molecular ions are not observed in the mass spectra of these compounds and their composition is uncertain,[1] but we have found the composition of prasinon B to approximate to $C_{43}H_{63}NO_{12}$.

The prasinons are weakly acidic and show enolic properties. Reduction of prasinon B over palladium–charcoal catalyst was accompanied by hydrogenolysis, giving a crystalline acidic product shown by mass spectrometry to have the composition $C_9H_{11}NO_5$, and a neutral amorphous product which gave a parent ion $C_{34}H_{56}O_5$. A C_{34} reduction product was also obtained by using Adams catalyst.

The physical properties of the *N*-containing moiety suggested the enolised *N*-2-(1,3-dioxocyclopentyl)succinamic acid structure (1; R = H) and this has been confirmed by synthesis. 2-Hydroxyiminocyclopentane-1,3-dione [2] was reduced with zinc and acetic acid and the resulting amine was treated *in situ* with succinic anhydride. The product (1; R = H) was identical with the hydrogenolysis product of prasinon B. The



(1)



(2)

methyl ester (1; R = Me) was identical with dihydroflavensomycinic acid which results from catalytic reduction of the product (2; R = Me) of methanolysis of the antibiotic flavensomycin, from *S. cavourensis*.[3-5] In

[1] S. J. Box, M. Cole, and G. H. Yeoman, *Appl. Microbiol.*, 1973, **26**, 699.
[2] A. Corbella, G. Jommi, G. Ricca, and G. Russo, *Gazzetta*, 1965, **95**, 948.
[3] L. Canonica, G. Jommi, F. Pelizzoni, and G. Giolitti, *Gazzetta*, 1961, **91**, 1306; L. Canonica, G. Jommi, and F. Pelizzoni, *ibid.*, p. 1315.
[4] L. Canonica, G. Jommi, and F. Pelizzoni, *Tetrahedron Letters*, 1961, 537.
[5] L. Canonica, A. Corbella, G. Jommi, F. Pelizzoni, and G. Scolastico, *Tetrahedron Letters*, 1966, 3031.

the previous work[4] a potentiometric titration of the acid (1; R = H) was carried out, but the compound was not characterised.

The mass spectrum of prasinon B showed fragment ions at *m/e* 538 and 211, of composition $C_{34}H_{50}O_5$ and $C_9H_9NO_5$, respectively. It seemed likely therefore that prasinon B, like flavensomycin, contained an *N*-(dioxocyclopentyl)fumaramate residue (2), a conclusion supported by the n.m.r. spectrum which showed signals at τ 7.4 (4 H, $CH_2{\cdot}CH_2$ in cyclopentanedione), 2.80 and 3.15 (2 H, AB, *J* 16 Hz), 1.5 (NH), and −3.2 (enolic OH), also present in the spectra of prasinon A, flavensomycin, and flavensomycinic acid (2; R = Me). The mass spectra of prasinon A and flavensomycin also showed the same $C_9H_9NO_5$ fragment ion at *m/e* 211.

Prasinon B, like flavensomycin, readily undergoes methanolysis.[1] Specimens crystallised from carbon tetrachloride and, presumably, containing traces of acid, rapidly underwent decomposition, even at room temperature, yielding the carbon tetrachloride-insoluble acid (2; R = H).

Flavensomycin, which is known to have insecticidal properties,[6] has been assigned the composition $C_{38}H_{58}N_2O_{10}$,[3] although an antibiotic resembling flavensomycin, isolated from *S. griseus* by a different group of workers,[7] has been reported[8] to have the composition $C_{47}H_{67}NO_{14}$. The mass spectrum of flavensomycin showed a parent ion at *m/e* 568 ($C_{34}H_{52}O_6$) differing in composition by OCH_2 from the parent ion of prasinon B. This difference may be correlated with signals in the n.m.r. spectrum at τ 6.35 and 6.75 from two OMe groups (*cf.* ref. 3) as compared with one (τ 6.8) in the prasinons.

The present work shows that among the metabolic products of the Actinomycetes a group of compounds exists containing the residue (2). Similar side chains have been reported for the antibiotics manumycin[9] and moenomycin;[10] and two 2-aminocyclopentanedione residues are present in limocrocin.[11]

EXPERIMENTAL

M.p.s were taken with a Kofler hot-stage apparatus and are corrected. I.r. spectra were determined for Nujol mulls, and u.v. spectra and optical rotations for solutions in methanol. N.m.r. spectra were obtained at 100 MHz for solutions in deuteriochloroform with tetramethylsilane as internal standard. Molecular weights were taken from mass spectra. Mass spectra at high resolution were recorded at 70 eV with a Varian CH5 double-focusing instrument interfaced with a Varian 620L computer. Merck G_{254} silica gel was used in t.l.c.

Prasinon B, $[\alpha]_D^{20}$ +30°, purified as described,[1] was repeatedly recrystallised from carbon tetrachloride giving citrine plates, m.p. 125° [Found: C, 55.7; H, 6.7; N, 1.7%; (dried at 80 °C) C, 64.8; H, 8.2; N, 1.9%; (ref. 1) C, 65.5; H, 8.2; N, 2.2%. $C_{43}H_{63}NO_{12}$,CCl_4 requires C, 56.2; H, 6.8; N, 1.5%. Calc. for $C_{43}H_{63}NO_{12}$: C, 65.7; H, 8.1; N, 1.8%], $\nu_{max.}$ 3 360br, 3 240, 1 725, 1 675, 1 615, and 1 550 cm^{-1} (in $CHCl_3$ 3 360, 1 720, 1 672, and 1 630 cm^{-1}), $\lambda_{max.}$ 249 and *ca.* 280 nm ($E^{1\%}_{1cm}$ 630 and 393), *m/e* 538.362 8 (calc. for $C_{34}H_{50}O_5$: 538.365 8), 506.338 1 (calc. for $C_{32}H_{46}O_4$: 506.338 6), and 211.048 9 (calc. for $C_9H_9NO_5$: 211.048 1), τ −3.3 (s, enolic OH), 1.2 (s, NH), 2.8br (1 H, s), 2.80 and 3.15 (2 H, AB, *J* 16 Hz, CO·CH:CH·CO), 3.5 (1 H, dd, *J* 14.5 and 10 Hz), 4.1 (1 H, s), 4.2 (1 H, s), 4.2 (s, OH), 4.7 (2 H, m), 5.0 (1 H, d, *J* 8 Hz), 5.2 (s, OH), 5.90 and 6.15 (2 H, AB, *J* 12 Hz, CH_2O), 6.0 (1 H, s), 6.75 (3 H, s, OMe), 7.4 (4 H, s, cyclopentanedione), 7.95—8.1 (9 H, 3 C:CMe), 7.95—9.3 (*ca.* 12 H), and 9.0—9.3 (15 H, 5 CMe) (on shaking with D_2O the signals at τ −3.3, 1.2, 4.2, and 5.2 were removed).

Prasinon B was insoluble in sodium hydrogen carbonate but dissolved in 2N-sodium hydroxide. In ethanol it gave a deep brownish-green colour with iron(III) chloride.

Prasinon A, $[\alpha]_D^{30}$ +16,[1] separated from acetone–light petroleum (b.p. 60—80 °C) as an amorphous powder, $\nu_{max.}$ 3 450, 3 240, 1 700, 1 670, 1 615, and 1 550 cm^{-1}, τ −3.1 (s, enolic OH), 1.6 (s, NH), 2.90 and 3.20 (2 H, AB, *J* 16 Hz, CO·CH:CH·CO), 3.5 (1 H, dd, *J* 15 and 10 Hz), 4.2 (2 H, m), 4.7—5.1 (3 H, m), 6.0—6.8 (6 H, m), 6.8 (3 H, s, OMe), 7.4 (4 H, s, cyclopentanedione), 8.0—8.2 (9 H, 3 C:CMe), 8.0—9.2 (m), and 8.8—9.2 (4—5 CMe), *m/e* 538.362 9 (calc. for $C_{34}H_{50}O_5$: 538.365 8) and 211.048 7 (calc. for $C_9H_9NO_5$: 211.048 1). Prasinon A was insoluble in sodium hydrogen carbonate and gave a brownish-green colour in ethanol with iron(III) chloride.

Authentic flavensomycin had m.p. 130°, $[\alpha]_D$ −22° (*c.* 0.322), and gave the same iron(III) colour reaction as the prasinons; τ −3.2 (s, enolic OH), 1.5 (s, NH), 2.80 and 3.12 (2 H, AB, *J* 16 Hz), 6.35 (3 H, s, OMe), 6.75 (3 H, s, OMe), and 7.4 (4 H, s), *m/e* 568.375 4 (calc. for $C_{35}H_{52}O_6$: 568.376 3), 525.321 5 (calc. for $C_{32}H_{45}O_6$: 525.321 6), and 211.047 7 (calc. for $C_9H_9NO_5$: 211.048 1).

Hydrolysis of Prasinon B.—The carbon tetrachloride solvate (11 mg), which had been stored for some weeks at room temperature, was heated at 100 °C for 5 min. It was then extracted with carbon tetrachloride and the insoluble portion (4 mg) crystallised from acetone giving the acid (2; R = H) (Found: M^+, 211.046 3. Calc. for $C_9H_9NO_5$: *M*, 211.048 1), identical in spectroscopic properties with authentic material (see below).

Reduction of Prasinon B.—(a) *Over palladium.* Prasinon B (4 mg) in ethyl acetate (2 ml) was hydrogenated over palladium–charcoal (5%; 5 mg) at room temperature during 5 h. The crude product was crystallised from methanol giving prisms, m.p. 175—180° and >300°, of the acid (1; R = H) (Found: N, 6.7%; M^+, 213.064 8. Calc. for $C_9H_{11}NO_5$: N, 6.6%; *M*, 213.062 7), identical in spectroscopic properties and in behaviour on heating with synthetic material (see below).

The residue from the mother liquor was subjected to preparative t.l.c. in benzene-ethyl acetate (4 : 1). Recovery of the material from a dark band (u.v. light), R_F 0.7, gave the product as a foam (Found: N, 0%; *m/e*, 544.407 5. Calc. for $C_{34}H_{56}O_5$: *M*, 544.412 6), $\lambda_{max.}$ 273 nm ($E^{1\%}_{1cm}$ 30.6).

(b) *Over platinum oxide.* Prasinon B (20 mg) was

[6] R. Craveri and G. Giolitti, *Nature*, 1957, **179**, 1307.
[7] C. I. Chacko and D. Gottlieb, *Phytopathology*, 1965, **55**, 587.
[8] D. Gottlieb in 'Antibiotics. I. Mechanism of Action,' ed. D. Gottlieb and P. D. Shaw, Springer, Berlin, 1967, p. 617.
[9] K. Schroder and A. Zeeck, *Tetrahedron Letters*, 1973, 4995.
[10] H. Brockmann, H-U. May, W. Lenk, and H. Brockmann, *Chem. Ber.*, 1969, **102**, 3217.
[11] R. Tschesche, D. Lenoir, and H. Weidenmuller, *Tetrahedron Letters*, 1969, 141.

hydrogenated over pre-reduced platinum oxide (10 mg) in acetic acid during 4 h at room temperature. The crude product was purified by preparative t.l.c. in benzene–ethyl acetate (4 : 1) giving an oily alcohol (11 mg) (Found: N, 0%; *m/e*, 552.475 8. Calc. for $C_{34}H_{64}O_5$: *M*, 552.475 3), $\nu_{max.}$ 3 520 and 1 730 cm^{-1}.

N-2-(1,3-*Dioxocyclopentyl*)*succinamic Acid* (1; R = H).—2-Hydroxyiminocyclopentane-1,3-dione [2] (127 mg) in glacial acetic acid (1 ml) was added dropwise to a stirred solution of succinic anhydride (0.1 g) in dioxan (3 ml) at room temperature while zinc powder (reagent grade; 1 g) was added in portions. After 3 h the mixture was filtered and the solvent removed from the filtrate *in vacuo*. The residual tar was dissolved in aqueous sodium hydrogen carbonate and combined with aqueous washings of the spent zinc. Coloured neutral products obtained by extraction of this solution with chloroform were discarded. The solution was then acidified with concentrated hydrochloric acid and extracted with ethyl acetate. The recovered product was purified by preparative t.l.c. on silica gel (20 × 20 × 0.05 cm) in di-isopropyl ether–formic acid–water (90 : 7 : 3). The material from a band at R_F 0.1 was extracted with warm water and the aqueous extract (pH 3) was then extracted with ethyl acetate. The product crystallised from ethyl acetate in needles, $\nu_{max.}$ 3 240, 3 170, 3 080, 1 715, 1 675w, and 1 590 cm^{-1}, or from methanol in prisms, $\nu_{max.}$ 3 400—2 500br, 1 720br, and 1 545 cm^{-1} of the *acid* (1; R = H) (5 mg), m.p. 188°, resetting at once to prisms, m.p. >300° (Found: C, 50.4; H, 5.4; N, 6.6%; M^+, 213. $C_9H_{11}NO_5$ requires C, 50.7; H, 5.2; N, 6.6%; *M*, 213), $\lambda_{max.}$ 259 nm (log ε 4.42), *m/e* 213, 195, 167, and 113.

The methyl ester formed prisms, m.p. 134° (lit.,[3] 135°), from benzene–hexane (1 : 1) identical in spectroscopic properties, $\nu_{max.}$ 3 250, 1 735, 1 615, and 1 550 cm^{-1}, τ −2.6 (enolic OH), 2.2 (NH), 6.3 (3 H, OMe), 7.3 (4 H), and 7.4 (4 H), with those reported [3] for the ester (1; R = Me). Compounds (1; R = H) and (1; R = Me) in ethanol gave greenish-brown colours with iron(III) chloride similar to those given by flavensomycin and the prasinons.

Flavensomycinic Acid (2; R = Me).—This crystallised from benzene in citrine prisms, m.p. 235° (lit.,[3] 232—233°), $\nu_{max.}$ 3 220, 3 075, 1 722, 1 690, 1 625, 1 602, and 1 565 cm^{-1}, τ −3.0 (s, OH), 1.6 (s, NH), 2.80 and 3.15 (AB, *J* 16 Hz), 6.22 (3 H, s, OMe), and 7.4 (4 H, s). It was prepared [5] by reduction of 2-hydroxyiminocyclopentane-1,3-dione with tin and hydrochloric acid and reaction of the resulting amine with methyl 3-(chloroformyl)acrylate.

The acid (2; R = H), prepared [3] by hydrolysis of the ester (2; R = Me) crystallised from ethyl acetate in citrine prisms, m.p. 259—260° (decomp.) [lit.,[3] 249—251° (decomp.)], $\nu_{max.}$ 3 223, 3 080, 1 700, 1 655, and 1 600 cm^{-1}.

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