Tetrahedron Letters, Vol.22, No.34, pp 3293 - 3296, 1981
Printed in Great Britain

0040-4039/81/343293-04$02.00/0

STRUCTURE ELUCIDATION AND SYNTHESIS OF (2S)-4-OXO-1-AZABICYCLO[3.3.0]OCTA-5,7-DIENE-2-CARBOXYLIC ACID, A NEW METABOLITE ISOLATED FROM STREPTOMYCES OLIVACEUS

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Summary: The structure of a new metabolite isolated from Streptomyces olivaceus has been shown to be (2S)-4-oxo-1-azabicyclo[3.3.0]octa-5,7-diene-2-carboxylic acid by means of its spectroscopic properties and total synthesis.

Recent reports from these laboratories have described the isolation and structure elucidation of several new fused bicyclic β-lactam antibiotics collectively known as the olivanic acids.[1] During the isolation of certain of these antibiotics from culture filtrate generated by fermentation of Streptomyces olivaceus using essentially the previously described conditions[2], a further metabolite with a distinctive absorption at $\lambda_{max.}$ (H_2O) 295 nm in the u.v. spectrum was detected. This chromophore remained unchanged in the presence of cysteine, a feature which indicates the absence of a β-lactam ring in the molecule. Subsequent isolation and characterisation was to prove that this metabolite was not a new olivanic acid, but possessed a novel dihydro-1H-pyrrolizine structure.

The metabolite was extracted and partially purified by methods based on anion exchange chromatography to yield a freeze-dried sodium salt, which was alkylated with methyl iodide to afford a pure mono methyl ester. The ester,[3] obtained as a colourless oil which formed a low-melting waxy solid on cooling, gave a molecular ion at m/e 179.0572 in the mass spectrum corresponding to a molecular formula of $C_9H_9NO_3$. Absorptions at 1750 and 1700 cm^{-1} in the i.r. spectrum suggested the presence of a second carbonyl function in the molecule. The p.m.r. spectrum ($CDCl_3$) of the compound revealed a single methyl ester signal (δ 3.79) and a non-equivalent methylene group at δ 3.25 (2H, AA'X) coupled to a methine proton at δ 5.07 (1H, dd, J 7.5 and 5.5 Hz). The remaining three protons at δ 6.47 (1H, dd, J 2.5 and 4 Hz), 6.68 (1H, dd, J 4 and 1 Hz) and 7.10 (1H, dd, J 2.5 and 1 Hz) were intercoupled and possessed the characteristics of a pyrrole ring system with substitution at the nitrogen atom and the α-position.[4] The ^{13}C n.m.r. spectrum[3] confirmed the presence of nine carbon atoms including the four pyrrole-carbon atoms and the second carbonyl group (δ 186.35 ppm). It was thus deduced that the fragments from which the molecule is composed are those shown in Figure 1.

Structures (1), (2), (3) and (4) represent the ways in which the molecular fragments can be assembled, but the last-named two structures could be eliminated on the basis of the u.v. and i.r. spectra of the ester. Compounds (3) and (4) are N-acylpyrroles[5] which would not possess the observed u.v. absorption (295 nm). They also contain a γ-lactam ring system as in the reported derivative (5)[6], the lactam carbonyl absorption of which appears at 1760 cm^{-1} in the

i.r. spectrum. The observed carbonyl absorption pattern (1750 and 1700 cm^{-1}) would therefore be inconsistent with these two structures. However, the 2,3-dihydro-1H-pyrrolizin-1-one (6)[7] possesses absorptions at $\lambda_{max.}$ (EtOH) 288 nm and $\nu_{max.}$ ($CHCl_3$) 1700 cm^{-1}, values very similar to those shown by the ester of the new metabolite.

Of the two remaining possible structures (1) and (2), the latter could be discounted on the basis of p.m.r. chemical shifts. In the dihydro-1H-pyrrolizine derivative (7)[8] the chemical shifts of the methylene protons adjacent to the nitrogen atom and the carbonyl moiety are δ 4.35 and 3.12, respectively, whilst in compound (8)[8] the p.m.r. shift of the methylene group is δ 4.55. The corresponding protons of the methyl ester of the new metabolite appear at δ 3.25, a value which, in comparison with the data for compounds (7) and (8), is only consistent with structure (1).

Thus, on the basis of spectroscopic evidence for its methyl ester, the metabolite from Streptomyces olivaceus was assigned the dihydro-1H-pyrrolizine structure (9). Other natural products with the same bicyclic nucleus include compound (10)[4], extracted from the hairpencil secretions of a Trinidad butterfly, and heliotridine (11),[8] one of a family of pyrrolizidine alkaloids from species of Senecio.

In planning a synthesis of compound (9) in order to confirm the structure of the new metabolite it was noted that the related derivatives (12) and (13) had been prepared by cyclisation of the acids (14) and (15), respectively, with polyphosphoric acid.[9] The N-substituted pyrrole derivatives (14) and (15) were synthesised by the reaction of diethoxytetrahydrofuran with the appropriate amine.[9] The diacid (16) was the desired intermediate for the present synthesis, but attempted condensation of aspartic acid with diethoxytetrahydrofuran (CH_3CO_2H, 80°) only resulted in poor yields (<10%) of this product. However, a similar reaction involving dl-dimethyl aspartate hydrochloride (CH_3CO_2H, Et_3N, 80°) afforded the dimethyl ester (17)[10] (m.p. 33 - 36°) (90%), which was readily saponified (NaOH, CH_3OH, H_2O) to the diacid (16) (95%). Attempts to cyclise the diacid (16) to the bicyclic derivative (9) with polyphosphoric acid or other strongly acidic reagents were unsuccessful, and it was consequently decided to prepare the mono-acid, mono-ester (18) which should be a direct precursor of the methyl ester (1).

The selective mono-demethylation of dimethyl esters with 1,1'-dimethylhydrazine has been reported.[11] When heated with the diester (17) (reflux, 6 h), this reagent effected mono-demethylation in the wrong sense to afford the trimethylhydrazinium salt (19) (m.p. 140 - 141°) (36%)[12] which was hydrolysed (5N HCl) to the single mono-acid (20) (m.p. 112 - 115°) (97%). The desired isomeric acid (18) was eventually obtained from the diacid (16) by first forming the internal anhydride (21) (dicyclohexylcarbodiimide, pyridine-acetone) which was treated, without purification, with methanol. Ring-opening of the anhydride occurred in a regioselective manner to afford largely the mono-acid (18) [32% from (16)] together with a trace of the regio-isomer (20) and the diester (17) (17%). Cyclisation of the acid (18) to the dihydro-1H-pyrrolizine (1) was achieved, albeit in poor yield, with polyphosphoric acid (90°), but a more efficient process (37%) utilised phosphorous pentoxide in toluene at 80°. The racemic product obtained from this synthesis was identical in all respects except optical rotation with

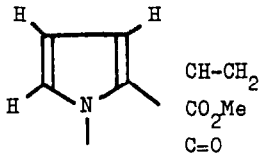
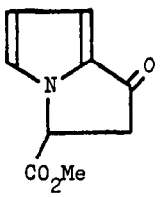
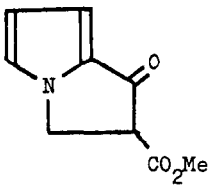


Figure 1



(1)



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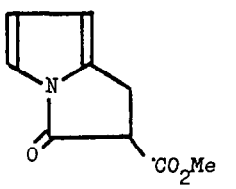
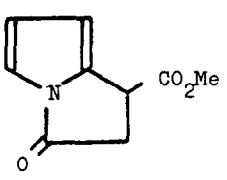
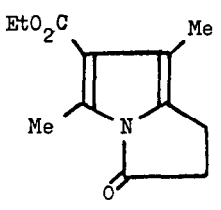


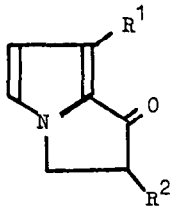
(3)



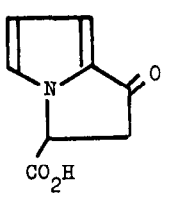
(4)



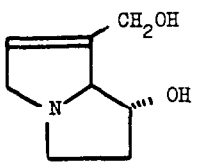
(5)



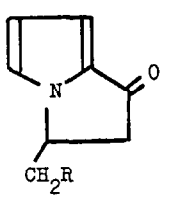
	R^1	R^2
(6)	H	H
(7)	CO_2Me	H
(8)	CO_2Me	CO_2Me
(10)	Me	H



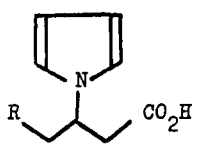
(9)



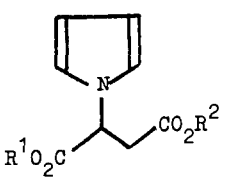
(11)



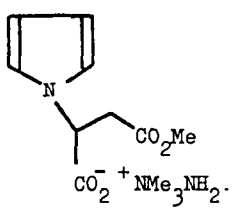
(12) R = H
(13) R = CO_2H



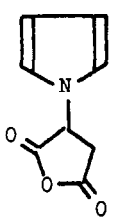
(14) R = H
(15) R = CO_2H



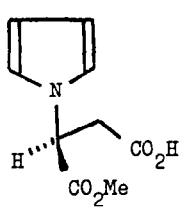
	R^1	R^2
(16)	H	H
(17)	Me	Me
(18)	Me	H
(20)	H	Me



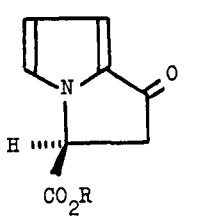
(19)



(21)



(22)



(23) R = Me
(24) R = H

the methyl ester of the natural product, thus confirming the structure (1) proposed on spectroscopic evidence.

The key intermediate having been identified, it was possible to simplify the synthetic procedure and at the same time determine the stereochemistry of the natural product. L-Aspartic acid was converted into its α-methyl ester,[13] which underwent condensation with diethoxytetrahydrofuran (CH_3CO_2H, 80°) to afford the pyrrole-derivative (22) (74%). Cyclisation of the acid (22) to the bicyclic derivative (23) was achieved with phosphorous pentoxide in toluene (37%). The optical rotation of the synthetic derivative was -95.5°, (c. 1 $CHCl_3$) compared to -101° (c. 0.83 $CHCl_3$) for the methyl ester of the natural product, thus establishing the configuration at C-2 of the latter as (S). Finally, the ester (23) was saponified (50%)[12] (NaOH, MeOH, H_2O) to complete the synthesis of (2S)-4-oxa-1-azabicyclo[3.3.0]octa-5,7-diene-2-carboxylic acid (24).[14] In contrast to the olivanic acids, the new metabolite showed no significant antibacterial activity or β-lactamase inhibitory properties.

References and Notes

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3. λ_{max} (EtOH) 286 nm; ν_{max} ($CHCl_3$) 1750 and 1700 cm^{-1}; δ^H ($CDCl_3$) 3.25 (2H, AA'X), 3.79 (3H, s, MeO_2C), 5.07 (1H, dd, J 7.5 and 5.5 Hz), 6.47 (1H, dd, J 2.5 and 4.Hz), 6.68 (1H, dd, J 4 and 1 Hz) and 7.10 (1H, dd, J 2.5 and 1 Hz); δ^C ($CDCl_3$) 43.21 (t), 53.18 (q), 55.39 (d), 108.23 (d), 117.37 (d), 123.93 (d), 132.44 (s) 169.34 (s) and 186.35 (s); (Found; M^+, 179.0572, $C_9H_9NO_3$ requires 179.0582).
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14. Partial racemisation occurred during the hydrolysis reaction.

(Received in UK 5 June 1981)