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# AGROPINE, A NEW AMINO ACID DERIVATIVE FROM CROWN GALL TUMOURS

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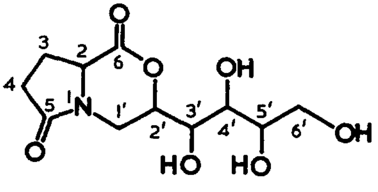
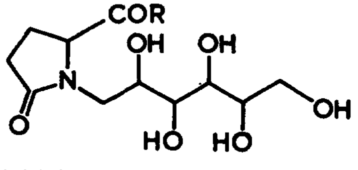
Summary: Agropine, an unusual compound produced in plant tumours was shown by mass spectrometry and proton and carbon-13 nmr spectroscopy to be a bicyclic derivative of glutamic acid and a hexitol sugar.

Crown gall tumours induced by certain strains of the bacterium Agrobacterium tumefaciens contain a number of structurally related unusual amino acid derivatives[1] whose synthesis is determined by bacterial DNA incorporated into the plant during initiation of the tumour. The structure of agropine[2], a novel compound of a different structural type, which may represent up to 7% of the dry weight of some tumours, is reported here.



(1) Agropine

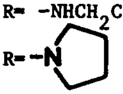


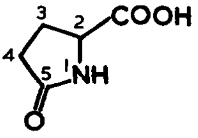
(3)(a) R=OH Agropinic acid

(b) R=OMe

(c) R= $-NHCH_2C_6H_5$

(d) R=





(2)

Agropine (1) was isolated by ion exchange chromatography of an aqueous extract of tumour

Table 1. $^{13}C$ Chemical shifts of agropine and related compounds (in ppm)

| | C-2 | C-3 | C-4 | C-5 | C-6 | C-1' | C-2' | C-3' | C-4' | C-5' | C-6' |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (1)[a] | 70.9 | 26.7 | 32.6 | 176.4[d] | 173.7[d] | 52.1 | 70.6[e] | 73.4[e] | 73.2[e] | 71.4[e] | 65.8 |
| (3a)[a] | 64.9 | 25.4 | 31.9 | 178.4[d] | 182.0[d] | 48.6 | 72.1[e] | 73.4[e] | 73.2[e] | 71.5[e] | 65.7 |
| (3c)[b] | 62.2 | 23.4 | 29.4 | 175.0[d] | 171.9[d] | 46.2 | 69.3[e] | 71.3[e] | 71.2[e] | 69.6[e] | 63.8 |
| (2)[a] | 56.8 | 25.2 | 30.1 | 176.8[d] | 182.6[d] | | | | | | |

[a] In acidified $D_2O$ - standard TSS

[b] In DMSO-$d_6$ - standard TMS

[d,e] Assignments may be transposed

Table 2. $^1H$ Chemical shifts of agropine and derivatives (360 MHz). (In $D_2O$ - (1) and (3a) or DMSO-$d_6$ - (3c))

| | 1'-$H_A$ | 1'-$H_B$ | 2'-H | 3'-H | 4'-H | 5'-H | 6'-$H_A$ | 6'-$H_B$ |
|---|---|---|---|---|---|---|---|---|
| (1) | 3.45 | 3.87 | 3.99 | 3.72 | 3.72 | 3.69 | 3.63 | 3.82 |
| | $J_{1'_A1'_B}$ | 15Hz | | $J_{2'3'}$ 9Hz | $J_{3'4'}$ ~0Hz | J[illegible]$_{5'}$ ~0Hz | $J_{6'_A6'_B}$ | 11.5Hz |
| | $J_{1'_A2'}$ | 8.5Hz | | | | | $J_{6'_A5'}$ | 6Hz |
| | $J_{1'_B2'}$ | 2.5Hz | | | | | $J_{6'_B5'}$ | 2.5Hz |
| (3a) | 3.01 | 3.84 | 3.77 | 3.62 | 3.71 | 3.66 | 3.58 | 3.78 |
| | $J_{1'_A1'_B}$ | 15Hz | | $J_{2'3'}$ 1Hz | $J_{3'4'}$ 9Hz | $J_{4'5'}$ 1Hz | $J_{6'_A6'_B}$ | 12Hz |
| | $J_{1'_A2'}$ | 9Hz | | | | | $J_{6'_A5'}$ | 6.5Hz |
| | $J_{1'_B2'}$ | 2.5Hz | | | | | $J_{6'_B5'}$ | 2.5Hz |
| (3c) | 2.75 | 3.83 | 3.4-3.7 | | | | | |
| | $J_{1'_A1'_B}$ | 15Hz | UNRESOLVED MULTIPLET | | | | | |
| | $J_{1'_A2'}$ | 9Hz | | | | | | |
| | $J_{1'_B2'}$ | 3Hz | | | | | | |

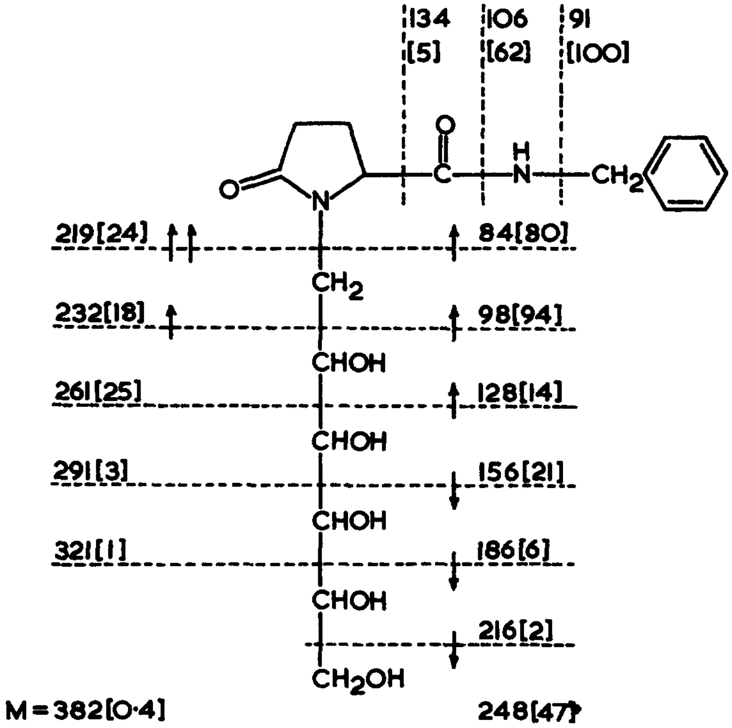
tissue and purified by preparative paper chromatography[2]. It was a somewhat intractable compound due to its hygroscopic nature and extreme solubility in water but was shown to be homogeneous by paper chromatography in two systems and by gas chromatography as the heptafluorobutyric n-propyl derivative[3]. Agropine did not react with ninhydrin but was detected on paper chromatograms with alkaline silver nitrate or lead tetraacetate-rosaniline reagents[4].

High resolution electron impact mass spectrometry indicated a molecular formula $C_{11}H_{17}NO_7$ ($M^+$ 275.1001) and fragmentation by losses of 18($H_2O$), 30($CH_2O$) and 31($CH_3O$). The base peak $^m/e$ 84 had the composition $C_4H_6NO$ which is a characteristic ion in the mass spectra of both glutamic acid and its lactam, 5-oxo-proline (2)[5]. In alkali, agropine was readily converted to agropinic acid (3a), $C_{11}H_{19}NO_8$ (MS f.d. M+1 294; e.i. base peak $^m/e$ 84) which yielded 5-oxo-proline on oxidation with permanganate or periodate.

The proton nmr spectrum of agropine (1) showed signals at $\delta$ 2.95 (2H, m, 3-$H_A$ and 3-$H_B$), 2.51 (1H, m, 4-$H_A$), 2.10 (1H, m, 4-$H_B$) and 4.53 (1H, dd J 10 and 4Hz, 5-H) characteristic of a 5-oxo-proline residue; corresponding signals were present in the spectrum of agropinic acid (3a). Similarly shifts of signals in the $^{13}C$ nmr spectra of (1) and (3a) were close to those of 5-oxo-proline (2) (Table 1). The remaining eight non-exchangeable protons in agropine were between $\delta$ 3.45 and 3.87 (Table 2) and, from a detailed analysis of the 360 MHz spectrum, could be assigned to a hexitol sidechain linked to the proline ring nitrogen.

The molecular formula of agropine requires it to be bicyclic and a lactone ring closure on to the hexitol side chain at C-2' was indicated by the chemical shift differences between agropine and agropinic acid of protons on C-1' and C-2' (Table 2). The difference between agropine and agropinic acid was supported by high voltage paper electrophoresis (pH3 and 10)-agropine behaved as a very weak base whereas agropinic acid behaved as a monocarboxylic acid.

The chemical reactions of agropine were consistent with a lactone structure. Reaction with MeOH/HCl gave a methyl ester (3b). Reaction with benzylamine in the presence of ammonium chloride gave a crystalline benzylamide derivative (3c) $C_{18}H_{26}N_2O_7$ (microanalysis and $M^+$ 382.1731), microscopic needles, m.p. 243-245°; $(\alpha)_D$+9°. Agropine consumed approximately 3 molar equivalents of periodate whereas the benzylamide (3c) consumed exactly 4 moles under the same conditions. The benzylamide (3c) and a similar pyrrolidide derivative (3d) obtained from agropine by a conventional method[6] gave mass spectral support for the proposed structure. A mass spectral fragmentation scheme of (3c) is given below.



Figures in parentheses are % relative intensity

The biosynthesis and biological activity of agropine are being investigated.

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