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ASPARAGUSIC ACID, DIHYDROASPARAGUSIC ACID AND S-ACETYLDIHYDROASPARAGUSIC ACID, A NEW PLANT GROWTH INHIBITORS IN ETIOLATED YOUNG ASPARAGUS OFFICINALIS.

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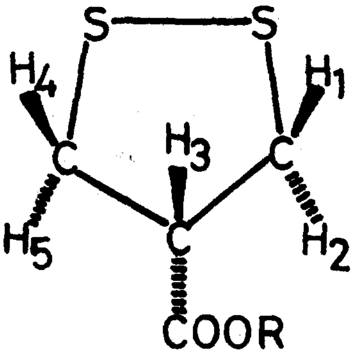
In the course of investigating the plant growth regulating substances from extracts of etiolated young asparagus (Asparagus officinalis L.), we isolated three compounds which apparently inhibited the growth of lettuce, here named asparagusic acid (I), dihydroasparagusic acid (III), and S-acetyldihydroasparagusic acid (V) respectively. This work describes the isolation and characterization of these compounds.[1]

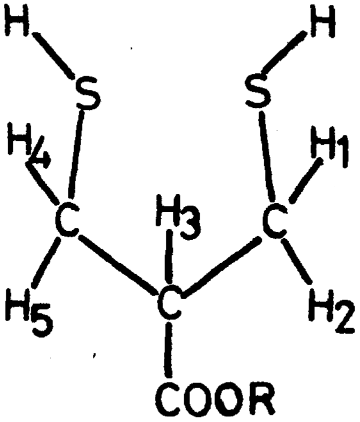
Asparagus officinalis L. is cultivated widely in the northern part of Japan, Tohoku and Hokkaido districts. The fresh etiolated tissues of asparagus were extracted with methanol and the methanol extracts were fractionated as follows by monitoring with lettuce growth. The aqueous methanol concentrate was acidified to pH 2.0 with diluted HCl and extracted with ether 3 times. The ether concentrate was extracted with 5 % aqueous sodium bicarbonate. The alkaline phase was, after acidified to pH 2.0, extracted with ether 4 times, followed by evaporation of ether. The ether concentrate was chromatographed on preparative TLC (silica gel, toluene: ethyl formate: formic acid = 5: 4: 1). The portion of Rf 0.65 was taken out of thin-layer plates, then extracted with methylene chloride. From the extract, yellow prisms of asparagusic acid (I) was obtained.

Asparagusic acid, mp 75.7-76.5° (from benzene-cyclohexane), has molecular formula $C_4H_6O_2S_2$ which was confirmed by high resolution mass spectrometric study of the methyl ester (II) $C_5H_8O_2S_2$ (M^+ 163.9972). The mass spectral fragmentation pattern of II exhibited strong peaks at m/e 164 (M^+, base peak), 133 ($M-OCH_3$), 132 (M-S), 105 ($M-COOCH_3$), 104 ($M-COOCH_3-H$), 86, 59, and 41.

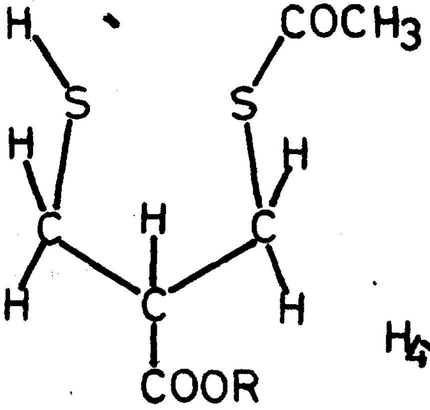
The IR spectrum of I showed a characteristic absorption at 1700 and 3500-2500 cm^{-1} for a carboxyl group. Although normal 60 MHz NMR spectrum of II in C_6D_6 showed only two signals at 2.60-3.20 (five protons) and 3.25 ppm (OCH_3), addition of 2.6 mole equivalents of Eu $(DPM)_3$ in the 100 MHz NMR of II in C_6D_6 caused the signals due to eight protons to separate into four groups which were assigned as follows. Two double doublets appear at 4.75 ppm (H_1 + H_4) and 6.77 (H_2 + H_5), a quintet at 7.00 (H_3), and a sharp singlet at 7.25 ($COOC\underline{H_3}$) with the coupling constants of $J_{1,3}$=7.2, $J_{2,3}$=5.7, and $J_{1,2}$=11.5 Hz, respectively. All the above spectral data can be accommodated in structure I[2] for asparagusic acid (1,2-dithiolane-4-carboxylic acid), which was confirmed by synthesis.[5]



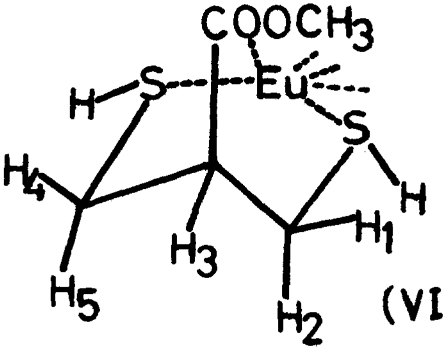
(I) R=H
(II) R=CH_3



(III) R=H
(IV) R=CH_3



(V) R=H
(VI) R=CH_3



(VII)

From the portion of Rf 0.55, dihydroasparagusic acid (III), $C_4H_8O_2S_2$, mp 59.5-60.5°, ν_{max}^{neat} 3500-2500, 2575, 1700, 1255, 910 cm^{-1} was obtained, which was methylated with diazomethane to give a methyl ester (IV). The 60 MHz NMR spectrum of IV (in $CDCl_3$) exhibited the signals at 1.50 (SH x 2, t), 3.00 (H_1+H_2+H_4+H_5, dd), 3.05 (H_3, quin), and 3.80 ppm ($COO\underline{CH_3}$, s) with the coupling constants of $J_{1,3}=J_{2,3}=6.4$ and $J_{1,SH}=J_{2,SH}=8.6$. Furthermore, addition of the europium shift reagent (2.7 mole equivalents of Eu $(DPM)_3$) in CCl_4 caused the changes of coupling modes of the signals accompanied with considerable shifts. A triplet appears at 3.21 ppm (SH x 2), two double double doublets at 5.58 (H_2 + H_5) and 5.91 (H_1 + H_4)[*2], a quintet at 6.96 (H_3), and a sharp singlet at 7.99 ($COO\underline{CH_3}$) with the coupling constants of $J_{1,SH}=J_{2,SH}=8.6$, $J_{1,2}=13.8$, $J_{2,3}=6.0$, $J_{1,3}=5.2$ Hz respectively. The extremely low field shift of methyl of COOMe group as well as changes of coupling mode and chemical shifts of other signals suggest that the europium atom was coordinated rigidly by carbomethoxyl and two thiols groups and VII is assumed as one of the possible complexes. A mass spectrum of IV showed strong peaks at 166 (M^+), 135 ($M-OCH_3$), 134 (M-S), 119 ($M-CH_2SH$), 107 ($M-COOCH_3$), 106 ($M-COOCH_3-H$), 100 ($M-(SH)_2$), 87 (base peak), 74, and 73. From these spectral data dihydroasparagusic acid (III) should be assigned β,β'-dimercaptoisobutyric acid, which was confirmed by synthesis (reduction of asparagusic acid with zinc in NH_4OH).

From the portion of Rf 0.70, S-acetyldihydroasparagusic acid (V) was isolated, which was methylated with diazomethane to give a methyl ester (VI). The mass spectrum of VI showed a molecular peak at m/e 208, with significant peaks at 207 (M-H), 177 ($M-OCH_3$), 165 ($M-COCH_3$), 164 ($M-COCH_3-H$), 148 ($M-COOCH_3-H$), 133 ($M-COCH_3-S$), 132 ($M-COCH_3-H-S$) and 43 (base peak). The IR spectrum of VI displayed the presence of characteristic carbonyl absorption of S-acetyl and carbomethoxyl groups at 1698 and 1740 cm^{-1} respectively.

These physical data suggested that V is β-S-acetyl-β'-mercaptoisobutyric acid, which was ascertained by comparison with a specimen prepared by reductive acetylation of asparagusic acid with zinc dust in acetic anhydride.

Asparagusic acid, dihydroasparagusic acid, and S-acetyldihydroasparagusic acid are very effective plant growth inhibitors, thus inhibited completely the growth of root and hypocotyl of lettuce at 6.67×10^{-4} M. The growth inhibiting activity of these compounds was very similar to that of abscisic acid.[7] The biological actions of I, III, and V are now actively investigated as compared with α-lipoic acid[8] which is known to participate the transfer of acyl group.

[*2] The assignment of these protons is not certain. The opposite i.e., 5.58 (H_1 + H_4) and 5.91 (H_2 + H_5) is almost equally possible.

Acknowledgement

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