

ASPARAGUSIC ACID-S-OXIDES,  
NEW PLANT GROWTH REGULATORS  
IN ETIOLATED YOUNG ASPARAGUS SHOOTS

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In a continuing study on the plant growth regulating substances in etiolated young asparagus (Asparagus officinalis L.), we have recognized two active compounds together with three acids, asparagusic, dihydroasparagusic, and S-acetyldihydroasparagusic acids, which were reported previously.<sup>1,2</sup> We now wish to report the isolation, structural elucidation and confirmation of the structure of the new compounds which similarly showed inhibitory effects on the growth in lettuce, rice, radish, and other plants.

The crude acidic fraction of ether soluble part from methanol extracts of etiolated tissues of asparagus shoots were separated by preparative TLC (silica gel; toluene: ethyl formate: formic acid=5: 4: 1) and the activities of each fraction were monitored with the germination assay of lettuce seeds. From active portions of Rf 0.31 and 0.25, compounds (I), C<sub>4</sub>H<sub>6</sub>O<sub>3</sub>S<sub>2</sub>, mp 127-128.5°, and (III), C<sub>4</sub>H<sub>6</sub>O<sub>3</sub>S<sub>2</sub>, mp 118.5-120°, were obtained as white crystallines, each of which was methylated with diazomethane to give a methyl ester (II), and (IV), respectively. \*2

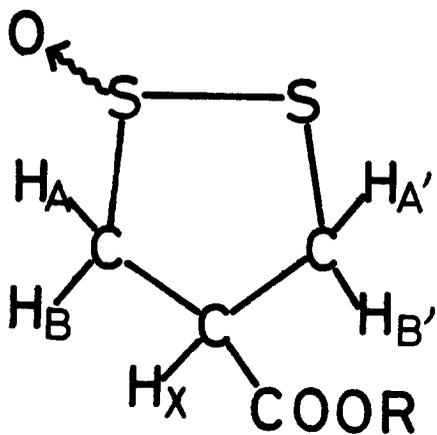
The physical evidence of these acids and esters was quite similar and suggested that I and III were isomeric carboxylic acid containing a sulfinyl group in a molecule. Thus, both I and III absorbed at  $\lambda_{\max}^{\text{MeOH}}$  248 nm (log $\epsilon$  3.1)<sup>4</sup> and the corresponding methyl esters showed a molecular peak at m/e 180 with significant peaks at 164 (M-0), 149 (M-OCH<sub>3</sub>), 148 (M-S, base peak), 147 (M-S-H), and 131 (M-SO-H) in the mass spectra. The major IR bands of both esters were observed at 1730, 1440, 1220, 1070, and 850 cm<sup>-1</sup>.

The 100 MHz NMR spectra of each ester showed an ABX and A'B'X patterns, i.e., clear multi-

plet of Hx of II at 4.23 ppm with  $J_{AX}=4.5$ ,  $J_{BX}=12.0$ ,  $J_{A'X}=7.0$ ,  $J_{B'X}=10.0$  and Hx of IV at 3.82 ppm overlapping with  $-\text{COOCH}_3$  with  $J_{AX}=6.7$ ,  $J_{BX}=4.2$ ,  $J_{A'X}=5.6$ ,  $J_{B'X}=4.7$ . Addition of a shift reagent [0.413 mole equiv of  $\text{Eu}(\text{DPM})_3$ ] caused the complete separation of the chemical shifts of each proton in both esters and Hx of II shifted to 8.28 ppm with  $J_{AX}=4.5$ ,  $J_{BX}=11.5$ ,  $J_{A'X}=7.0$ ,  $J_{B'X}=9.9$ , while Hx of IV appeared at 5.77 ppm with  $J_{AX}=7.5$ ,  $J_{BX}=4.7$ ,  $J_{A'X}=6.5$ , and  $J_{B'X}=5.5$ . On irradiation at Hx (8.28 ppm) of II, the spectrum changed to two pairs of AB type at 7.49 ppm and 5.96 ppm with  $J_{AB}=13.0$  and 6.31 ppm and 5.63 ppm with  $J_{A'B'}=10.5$ , respectively.

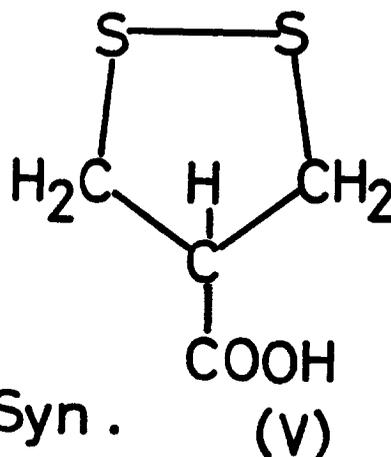
The spectral evidence described above and coexistence of V in the same plant indicate that I and III are stereoisomer of 1,2-dithiolane-4-carboxylic acid-1-oxide, which was confirmed by synthesis.<sup>3</sup> Synthetic asparagusic acid (V)<sup>1</sup> was oxidized<sup>3</sup> with sodium metaperiodate, under the conditions to prevent further oxidation to sulfone, to afford a mixture of I and III with the ratio of 77 to 23. Other oxidizing agents gave the same mixture, always providing a marked predominance of compound (I).

The stereochemistry of S-oxide with respect to COOH was assigned on the basis of 1) Rf value on chromatography, 2) predominant formation of I with oxidizing agents, and 3) the relative degree of down field shifts by the addition of  $\text{Eu}(\text{DPM})_3$ .<sup>5</sup> Comparison of these observations suggested that I and III are asparagusic acid-anti-S-oxide (anti-1,2-dithiolane-4-carboxylic acid-1-oxide), and its syn isomer, respectively.



(I)  $R=H$  Anti, (III)  $R=H$  Syn.

(II)  $R=CH_3$  Anti, (IV)  $R=CH_3$  Syn.



Although the relatively lower concentration ( $6.67 \times 10^{-6}M$ ) of the asparagusic acid-S-oxide slightly inhibited the root growth in lettuce, it completely inhibited the root and hypocotyl growth at the concentration of  $6.67 \times 10^{-4}M$ . The growth inhibiting activity of each isomer was almost equal to that of asparagusic acid. This is the first example of the isolation and characterization of I and III from natural source and add another example on the occurrence of S-oxides from natural sources, i.e., S-alkyl and S-allyl-L-cysteine sulfoxides in allium<sup>6</sup> and brassica<sup>7</sup> species as well as brugierols in Bruguiera conjugata Merr.<sup>8</sup>

#### References

- \*1 To whom all correspondences should be addressed.
- \*2 The contents of I and III from 100 Kg of fresh tissues of asparagus were 10 mg and 4 mg, respectively.
- \*3 Although  $[\alpha]_D$  was not measured owing to limited amounts of natural oxides which are expected to be optically active, both IR (neat) and Mps of synthetic specimens were identical with those of natural products. This evidence suggests natural oxides exist as racemic form.
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