was no significant difference in tolerance development in STR on administration of morphine (10 mg/kg), either twice a day or four times a day. Hence, it is possible that the greater the tissue concentration of morphine given to the mice, the faster the tolerance development in STR. Furthermore, tolerance in STR was observed clearly on daily injection of morphine into the cerebral ventricle in mice. This finding supports the view that the tolerance in STR may be developed in the CNS in mice.

It has previously been reported that an increase in 5-HT activity in the CNS prevents STR.[7a] It has also been suggested, on the contrary, that 5-HT may be important for tolerance development induced by morphine in contrast to other proposed neurotransmitters, *i.e.*, acetylcholine, dopamine and norepinephrine.[14] The cortical 5-HT content in tolerant mice was decreased, compared to that in non-tolerant mice, without any change in cortical 5-HIAA content. There was no significant change in 5-HT or 5-HIAA content in the mesencephalon plus diencephalon, pons plus medulla oblongata, or in the thoracic or lumbosacral cord. Therefore, the marked inhibition of STR by L-5-HTP in tolerant mice compared to that in non-tolerant mice might be attributed to post-synaptic hyperexcitability[14,15] induced by the decrease in the activity of cortical 5-HT neurons. Further experiments are in progress.

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Evidence for the Presence of O-Acetylserine in *Citrullus vulgaris*[1]

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O-Acetylserine (**2**), a substrate for cysteine synthase and β-substituted alanine synthase(s) in plants, was identified after transformation to N-acetylserine in the greenish-white epicarp and the reddish mesocarp of the intact fruits of water-melon (*Citrullus vulgaris*) in concentrations of 1.05×10^{-6}% and 0.98×10^{-7}%, respectively. No endogenous N-acetylserine was detected by the same procedure.

Keywords——amino acid; O-acetyl-L-serine; N-acetylserine; serine; β-substituted alanine; enzyme; β-substituted alanine synthase; *Citrullus vulgaris*; water-melon

Our recent reports have shown that O-acetyl-L-serine (**2**) has an important role as a key intermediate in the biosyntheses of β-substituted alanines, such as β-(pyrazol-1-yl)-L-alanine

1) This work was presented at Meeting of Kanto Branch, Pharmaceutical Society of Japan, Tokyo, October, 1977, and was also briefly quoted in a reference cited in Ref. 4 of this paper.
2) Location: *1-33 Yayoi-cho, Chiba-shi, Chiba, 260, Japan.*

(**4**),[3] O-ureidoserine,[4] β-uracilylalanines[5] and similar compounds,[6–10] in higher plants. Although cysteine synthase present in higher plant extracts[11,12] also apparently utilizes O-acetyl-L-serine more readily than L-serine (**1**) as a sulfide acceptor,[13] the β-substituted alanine synthase (s) clearly appears to be specific for the O-acetyl-L-serine as a donor of the alanyl-moiety. However, O-acetyl-L-serine has not yet been isolated as a natural constituent, although it ought to be widely distributed in nature since the biosynthesis,[14,15] utilization,[3–10,16–19] and degradation (β-eliminase)[20] of O-acetyl-L-serine have already been demonstrated to occur as enzymic processes in microorganisms and higher plants.

This report presents preliminary evidence for the presence of O-acetylserine in the unripe fruits of water-melon (*Citrullus vulgaris*). It is already known that β-(pyrazol-1-yl)-L-alanine (**4**) occurs in large amounts in the pressed juice[21] and seeds[22] of water-melon, and seedlings of water-melon form the most active source of β-(pyrazol-1-yl)-L-alanine synthase,[3] which catalyzes the formation of **4** from **2** and pyrazole (**3**)[23] (Fig. 1). From the above results, unripe fruits of water-melon were chosen in an attempt to demonstrate that **2** occurs naturally in plants.

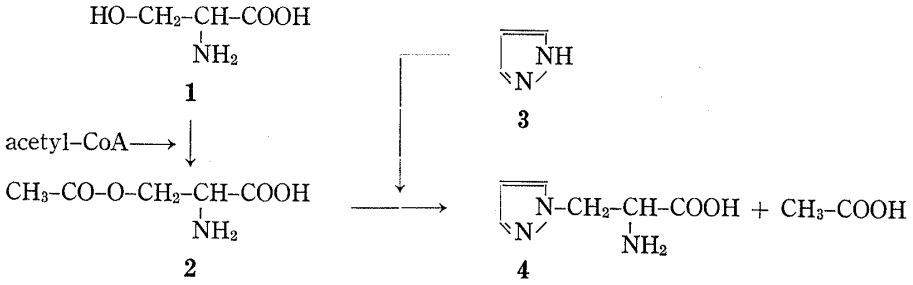

Fig. 1. Scheme for the Biosynthesis of β-(Pyrazol-1-yl)-L-alanine (**4**) by Enzymes in the Seedlings of Water-melon (*Citrullus vulgaris*) (Reference 3)

Pyrazole (3) has also been found in the seeds and seedlings of water-melon (reference 23).

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21) S. Shinano and T. Kaya, *J. Agric. Chem. Soc. Japan*, **31**, 759 (1957).
22) F.F. Noe and L. Fowden, *Biochem. J.*, **77**, 543 (1960).
23) The presence of free pyrazole has been confirmed in the seeds and seedlings of water-melon (T.A. LaRue and J.J. Child, *Phytochemistry*, **14**, 2512 (1975)).

Since **2** is converted readily to the N-acetylserine under alkaline conditions (pH above about 8.0) even at room temperature,[9,15,20,24,25] it was identified chromatographically as N-acetylserine in extracts of water-melon fruits. Extracts in 75% EtOH of the greenish-white epicarp and the reddish mesocarp of the unripe fruits of water-melon were brought to pH 10.0 by adding aq. NH_4OH and allowed to stand in a cold room for about 20 hr. After centrifugation, the extract was adjusted to pH 7.0 and applied to an Amberlite IRA-410 (acetate-form) resin column, which was eluted with 3—4.5% AcOH, giving fractions containing N-acetylserine. These fractions were applied to a Dowex 50W (H-form) resin column to separate N-acetylserine from acidic amino acids. The eluate from the Dowex 50W column was concentrated *in vacuo* and the presence of N-acetylserine was confirmed as an index of the original presence of **2** in the unripe fruits.

An aliquot of the 75% EtOH extract which had not been treated with NH_4OH was treated in the way described above and was used as a control sample.

The identity of the N-acetylserine was confirmed by its chromatographic behaviour in comparison with that of authentic material and by converting the N-acetylserine into serine by hydrolysis with 20% HCl, as described in "Experimental".

By the above method, more than 92—95% of O-acetylserine was obtained as N-acetylserine or serine in a recovery experiment. The amounts of O-acetylserine in the greenish-white epicarp and the reddish mesocarp of the intact fruits of water-melon were estimated to be 1.05×10^{-6}% and 0.98×10^{-7}%, respectively. No endogenous N-acetylserine was detected in the fruits of water-melon under the same experimental conditions.

These findings support the assumption of the widespread natural occurrence of O-acetyl-L-serine (**2**) as an active form of L-serine (**1**) utilized in the biosyntheses of cysteine[11,12,14–19] and its derivatives,[16,26] and of other type of β-substituted alanines.[3–10]

After the completion of this work, independent studies including ^{14}C-tracer experiments by Smith[27] with cultured tobacco cells have established the presence of O-acetylserine in this material.

Experimental

General Methods——Paper chromatograms (PC) were developed with the following solvent systems: 1, 2-propanol–HCOOH–H_2O (20: 3: 5, v/v); 2, 1-butanol–AcOH–H_2O (90: 10: 29, v/v); 3, 1-butanol–AcOH–H_2O (12: 3: 5, v/v). Spots were visualized with 0.5% ninhydrin in ethanol for amino acids and with 0.1% bromocresol green (BCG) reagent for acids as chromogenic reagents. An automatic amino acid analyzer (Shibata model AA-500, Tokyo) was operated under the standard conditions (150 cm column, 50°, 0.2 N Na citrate buffer, pH 3.25, flow rate 0.5 ml/min) as described in previous papers.[3–10]

Isolation of N-Acetylserine derived from O-Acetylserine (2)——The greenish-white epicarp (1.1 kg) or the reddish mesocarp (3.25 kg) of unripe fruits of water-melon (*Citrullus vulgaris*) was homogenized in 3 volumes of EtOH at 3—5°. The slurry was immediately brought to pH 10.0 by adding aq. NH_4OH, and then allowed to stand in a cold room. After about 20 hr, the alkaline slurry was adjusted to pH 7.0 with HCl and insoluble materials were removed by centrifugation.

The supernatant was applied to a column of Amberlite IRA-410 (acetate-form, 5×90 cm). The column was thoroughly washed with water to remove non-anionic material, including endogenous serine, and then eluted with 1—6% AcOH: N-acetylserine was mainly eluted with 3—4.5% AcOH. The eluates containing N-acetylserine were quickly concentrated *in vacuo* (a very small amount of N-acetylserine was converted to O-acetylserine and serine at this stage because of the acidic conditions) and the residue, adjusted to pH 5.5—6.0, was applied to a column of Dowex 50W (H-form, 2.4×30 cm) to separate N-acetylserine from acidic amino acids: N-acetylserine was not retained by the resin and was collected in the effluent. The fraction containing N-acetylserine and other organic acids was concentrated at low temperature and used

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as a sample (I) to demonstrate the presence of N-acetylserine. An original 75% EtOH homogenate which had not been adjusted to pH 10.0 with aq. NH_4OH was treated separately with Amberlite IRA-410 (acetate-form), followed by Dowex 50W (H-form) column, in the same way as that described above, and was used as a control sample (II).

Identification of N-Acetylserine and Serine derived from O-Acetylserine (2)——N-Acetylserine was first detected by PC of the above sample (I) by comparison with authentic material prepared from **2** by alkaline treatment,[24,28,29] using BCG reagent and ninhydrin, after hydrolysis of the BCG-positive compound on the PC into serine with 1% HCl by a modification of the method of Smith *et al.*.[15] The *Rf* values determined for N-acetylserine in solvents 1, 2 and 3 were 0.75, 0.50 and 0.57, respectively.

Further confirmation that the BCG-positive compound was N-acetylserine was obtained, after hydrolytic cleavage of the compound to serine, by amino acid analyzer and PC in 5 different solvent systems:[3-10] Sample (I) was streaked across sheets of Toyo No. 50 filter paper and the chromatograms were developed in solvent 3 for 20 hr. The BCG-positive bands corresponding to N-acetylserine were cut from each chromatogram, and the compound was eluted with 1% NH_4OH–MeOH. After removal of the solvent *in vacuo*, the residue was hydrolyzed to serine with 20% HCl at 105° for 2.5 hr. The hydrolysate was evaporated to dryness *in vacuo* and passed through a column of Dowex 50W (H-form, 1.2×20 cm). The column was eluted with 2—3% NH_4OH, the eluate was evaporated to dryness *in vacuo*, and the residue after hydrolysis was shown to be serine by PC and amino acid analyzer.

These properties of the BCG-positive compound are in agreement with those observed by Fujiwara *et al.*[24] and by Smith *et al.*[15,27] for authentic N-acetylserine.

An amino acid analyzer was used to determine the content of O-acetylserine, as serine, in the intact fruits: O-acetylserine was found in the greenish-white epicarp and the reddish mesocarp of the unripe fruits of water-melon in amounts of about 1.05×10^{-6}% and 0.98×10^{-7}%, respectively. Recovery of O-acetylserine by this method was in the range of 92—95%. Endogenous N-acetylserine was not found under the same experimental conditions.

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