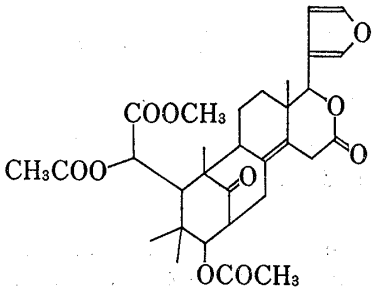
for steroids and triterpenes. These specimens were collected from various parts of west Malaysia. The plant extracts were prepared and chemical tests carried out according to the methods previously described.[2]

Detailed chemical studies of the seeds of *Swietenia macrophylla* grown locally has resulted in the isolation of a new compound, switenolide diacetate, which has not been previously reported in the seeds from this plant.[5]



switenolide diacetate

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5) K.C. Chan, T.S. Tang, and H.T. Toh, *Phytochemistry*, **15**, 429 (1976).

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L-Serine O-Sulphate Lyase, a New Enzyme in Extracts from Higher Plants[1]

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L-Serine O-sulphate (L-SOS) lyase, an enzyme capable of degrading L-SOS to pyruvate, ammonia and sulfuric acid, was newly found in extracts from higher plants. The optimum pH for the L-SOS lyase in higher plants differ from that for the enzyme(s) in animals and micro-organisms in the same buffer. The distribution and some properties of the L-SOS lyase in higher plants are described.

Keywords——amino acid; serine O-sulphate; O-acetyl-L-serine; enzyme; L-serine O-sulphate lyase; O-acetyl-L-serine lyase; O-acetyl-L-serine sulfhydrylase; Leguminosae; Liliaceae; Cruciferae

In recent years L-serine O-sulphate (I) has been implicated as an intermediate in the biosynthesis of cysteine derivatives such as S-methylcysteine and S-allylcysteine by extracts in *Leucaena*, *Albizzia*, *Citrullus* and *Allium* seedlings.[3] It is tentatively found in our laboratory that the enzyme which utilize O-acetyl-L serine and L-serine O-sulphate as a substrate for S-alkyl-cysteine formation in a number of higher plants are different.[4]

1) This work was presented at the 19th Kanto Branch Meeting of the Pharmaceutical Society of Japan at Chiba, November 15, 1975, Meeting Abstracts, p. 59.
2) Location: *1-33 Yayoi-cho, Chiba-shi, 280, Japan.*
3) I. Murakoshi, A. Yamazaki, and J. Haginiwa, presented at the 92th Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April 7, 1972. Meeting Abstracts, II. p. 260.
4) I. Murakoshi, F. Kato, and J. Haginiwa, *Chem. Pharm. Bull.* (Tokyo), **21**, 919 (1973); *idem*, *ibid.*, **22**, 473 (1974).

The present note reports the occurrence of a new enzyme, L-serine O-sulphate (L-SOS) lyase, in extracts from a number of higher plants, The enzyme cleaves L-SOS stoichiometrically to yield pyruvic acid, ammonia, and sulfuric acid as shown in Fig. 1.

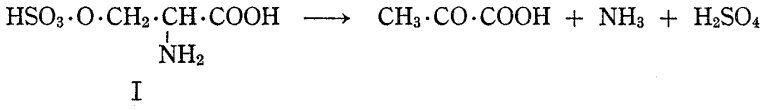

Fig. 1. Scheme for the L-Serine O-Sulphate Lyase

The distribution and some properties of L-SOS lyase in higher plants are described.

Materials and Methods

Chemicals——The O-sulphate esters of L- and D-serine, and of L-threonine were prepared as K-salt essentially by the method described by Dodgson, *et al.*[5] (yield: 35—45%). D-Serine O-sulphate was predominantly racemized with ease during the preparation when the time taken to reach the form of K-salt is longer even at room temperature. D-Serine O-sulphate, $[\alpha]_D^{23}$ +8.7 (c=1.0, H_2O).

Plant Materials——The seedlings of *Phaseolus radiatus*, *Pisum sativum*, *Brassica campestris*, *Raphanus sativus* and *Fagopyrum esculentum* had been grown in moistened vermiculite in the dark for 3—4 days at 30°, and *Mimosa pudica*, *Thermopsis chinensis*, *Lupinus luteus*, *Citrullus vulgaris*, *Leucaena leucocephala*, *Lathyrus odoratus*, *Mucuna capitata*, *Sophora flavescens*, *Robinia pseudo-acacia* and *Thermopsis fabacea* for 5—6 days at 30°. *Allium sativum* and *Allium cepa* bulbs purchased at local markets were kept at 30° for 1—2 days before extraction. After harvest, the testas were removed and then the seedlings or the bulbs were cooled at 0° for 60 min before the following operations.

Enzyme Preparation——All the following operations were carried out in the cold. The seedlings or bulbs were homogenized with 0.2 M Tris–HCl, pH 9.0, containing 0.2% (v/v) 2-mercaptoethanol in a mortar with silica sand using 0.5 ml buffer for each gram of plant materials. The clear supernatant solution recovered by centrifugation at 25000 g for 30 min was passed through a column of Sephadex G-25 (fine) to obtain an enzyme solution free from low mol.wt.substances as previously described.[6-8] The eluted protein was used as the source of enzyme activity.

Assay Procedures——The standard incubation mixtures were performed at 30° and contained 5 μmol of L-serine O-sulphate and 0.2 ml of enzyme solution (containing 0.5—2 mg of the soluble protein from 1.5 g fresh weight of plant materials) in a final volume of 0.4 ml. The pH of the incubation mixtures were normally maintained at pH 8.9—9.0 by 0.1 M Tris–HCl buffer. In the control samples boiled enzyme was used. The incubation was terminated by the addition of 0.6 ml of 25% trichloroacetic acid. The precipitate was removed by centrifugation and aliquots of the clarified solution were assayed for pyruvate by the total keto acid method of Friedemann and Haugen.[9] In some instances the liberated pyruvate was estimated by using a coupled assay procedure employing lactate dehydrogenase.[10] In balance studies the ammonia and sulfuric acid produced were measured colorimetrically with Nessler's reagent[11] and barium chloride-gelatin reagent,[12] respectively. Protein was determined by the method of Lowry, *et al.*[13]

Results

A preliminary survey of a number of plant species was carried out by preparing the homogenate in 0.2 M Tris-HCl buffer and testing these as sources of enzyme. L-SOS lyase activity was found in preparations from *Phaseolus*, *Citrullus*, *Fagopyrum*, *Pisum*, *Brassica*,

5) K.S. Dodgson, A.G. Lloyd, and N. Tudball, *Biochem. J.*, **79**, 111 (1961).
6) I. Murakoshi, H. Kuramoto, J. Haginiwa, and L. Fowden, *Phytochem.*, **11**, 177 (1972).
7) I. Murakoshi, F. Kato, and J. Haginiwa, *Chem. Pharm. Bull.* (Tokyo), **21**, 918 (1973); *idem*, *ibid.*, **22**, 473, 480 (1974); *idem*, *ibid.*, **23**, 285 (1975).
8) I. Murakoshi, F. Ikegami, F. Kato, J. Haginiwa, F. Lambein, L.V. Rompuy, and R.V. Parijs, *Phytochem.*, **14**, 1515 (1975).
9) T.E. Friedemann and G.E. Haugen, *J. Biol. Chem.*, **147**, 415 (1943).
10) N. Tudball, P. Thomas, and R. Bailey-Wood, *Biochem. J.*, **121**, 747 (1971).
11) D. Seligson and H. Seligson, *J. Lab. Clin. Med.*, **38**, 324 (1951).
12) K.S. Dodgson, *Biochem. J.*, **78**, 312 (1961).
13) O.H. Lowry, N.J. Rosebrough, A. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

Leucaena, *Allium*, and *Mimosa* seedlings tested as shown in Fig. 2. The later four were very active and the seedlings of *Leucaena leucocephala* (Leguminosae) became the plant of choice. Unless otherwise specified, the enzyme preparations obtained from *Leucaena* seedlings were used as the source of enzyme activity.

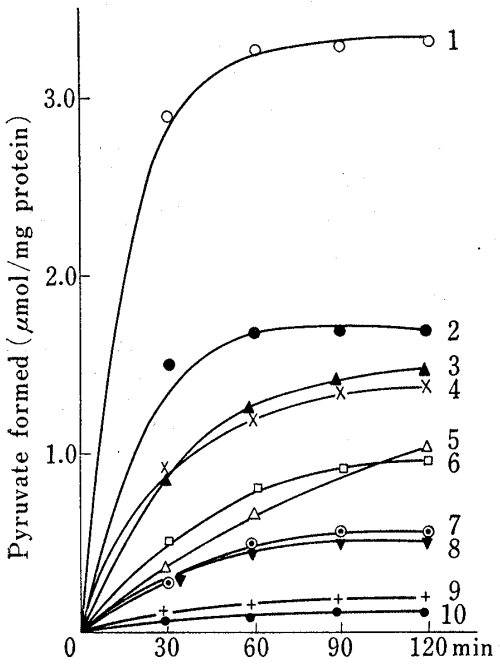

Fig. 2. The Comparative Activity of L-Serine O-Sulphate Lyase in Various Higher Plants

1: *Mimosa pudica*, 2: *Allium sativum*, 3: *Leucaena leucocephala*, 4: *Brassica campestris*, 5: *Pisum sativum*, 6: *Allium cepa*, 7: *Raphanus sativus*, 8: *Fagopyrum esculentum*, 9: *Citrullus vulfgaris*, 10: *Phaseolus radiatus*.

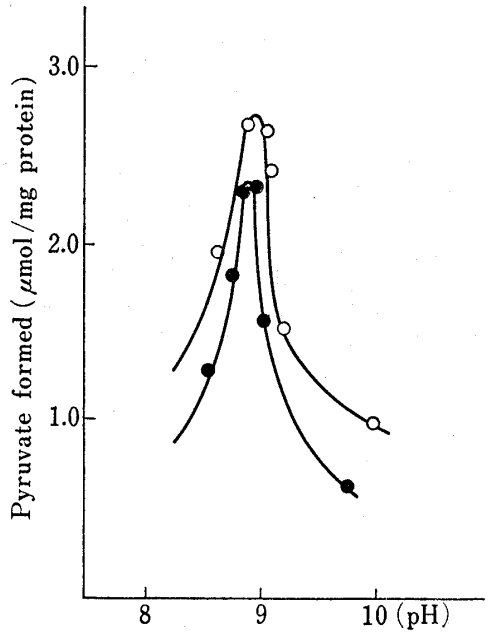

Fig. 3. The Effect of pH on the L-Serine O-Sulphate Lyase in the Extracts of *Leucaena* Seedlings

○—○, 0.1 M Tris-HCl buffer;
●—●, 0.1 M borate buffer.

The L-SOS lyase was shown to be quite specific for L-serine O-sulphate. D-Serine O-sulphate was not a substrate. No activity was detectable toward the O-phospho esters of L-serine and L-threonine. L-Threonine O-sulphate is degraded, but the activity shown toward this substrate is about 1/30 that shown toward the L-serine O-sulphate.

The optimum pH for the L-SOS lyase was 8.9 using 0.1 M Tris-HCl and borate buffer as shown in Fig. 3: the enzyme activity was higher in Tris-HCl than in borate.

Addition of exogenous pyridoxal phosphate up to 20 μg/ml to the reaction mixture had no effect on the L-SOS lyase but higher concentration (60 μg/ml) caused 25—30% inhibition. The optimum concentration of L-SOS was 188 mM under the experimental conditions.

The L-SOS lyase was resonably stable: when stored at 0° for 25 hr the remaining activity was about 84% of the activity associated with a freshly prepared enzyme fraction.

The rate of enzymatic degradation of L-SOS was constant for at least 30—45 min but the rate then decreased as shown in Fig. 2. The addition of hydroxylamine (25 mM), hydrazine (25 mM), and cyanide (1.25 mM) to reaction mixtures gave a complete inhibition of the L-SOS lyase activity. O-Phospho-L-serine is feeble noncompetitive inhibitor whereas D-SOS is weak competitive inhibitor.

Discussion

From the results presented here it can be concluded that higher plants contain an enzyme system capable of degrading L-serine O-sulphate (I) stoichiometrically to pyruvate, ammonia

and sulfuric acid in a similar manner as a L-serine O-sulphate degrading system known to be widely distributed in most animal phyla[14,15] and also in some microorganisms.[16] However, the optimum pH for the L-serine O-sulphate lyase in higher plants differ from that (pH 7.0) for the enzyme(s) in animals and micro-organisms in the same buffer.

Although L-serine O-sulphate has apparently not yet been found in nature, it appears that it ought to be. A similar problem is posed by the earlier finding in higher plant homogenates of an activity that deaminate O-acetyl-L-serine,[17] as well as O-carbamyl-L-serine in rat liver homogenates,[18] a compound not known to occur naturally.

At present, it is not certain whether the degradation of L-serine O-sulphate is catalyzed by the same enzyme as O-acetyl-L-serine lyase and O-acetyl-L-serine sulfhydrylase[19] described recently by Mazelis and Fowden,[20] and Yamagata, *et al.*,[21] respectively, and a more through examination using purified enzyme is being undertaken in our laboratory in an attempt to answer this question.

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14) N. Tudball and J.H. Thomas, in "Methods in Enzymology," edited by H. Tabor and C.W. Tabor, Vol. 17B, Academic Press, New York, 1971, p. 361.
15) Y. Noda, *J. Biochemistry*, **57**, 709 (1965).
16) T. Harada, *Biochem. Biophys. Acta*, **81**, 193 (1964).
17) O-Acetyl-L-serine clearly appears to be specific for heterocyclic β-sub.alanine synthetases (I. Murakoshi, *et al.*, Ref. 6, 7, 8).
18) A.J.L. Cooper and A. Meister, *Biochem. Biophys. Res. Comm.*, **55**, 780 (1973).
19) L-Serine O-sulphate is a substrate for the highly purified O-acetyl-L-serine sulfhydrylase from Baker's yeast but the rate of degrading activity is less than 1/5—1/6th of that of the O-acetyl-L-serine.
20) M. Mazeli and L. Fowden, *Phytochem.*, **11**, 619 (1972).
21) S. Yamagata, K. Takeshima, and N. Naiki, *J. Biochemistry*, **75**, 1221 (1974).