Absolute Configuration of L-Methionine Sulfoximine as a Toxic Principle in *Cnestis palala* (LOUR.) MERR.

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An unusual amino acid, L-methionine sulfoximine (1), has been isolated from the fresh seeds of *Cnestis palala* (LOUR.) MERR. [Connaraceae]. The absolute configuration of the natural sulfoximine (1) was confirmed to be 2(*S*)-methionine S(*S*)-sulfoximine [(2*S*,S*S*)-2-amino-4-(S-methylsulfonimidoyl)-*n*-butanoic acid] by comparison of the $[\alpha]_D$ value and IR spectrum with those of authentic samples obtained through the optical resolution of synthetic materials. Acute toxicity of the seeds of *C. palala* in a beagle dog was also studied.

Keywords *Cnestis palala*; Connaraceae; L-methionine sulfoximine; non-protein amino acid; absolute configuration acute toxicity

As a continuation of our screening program for physiologically active products in plants,[1–3] an unusual amino acid (**1**) has been isolated as a toxic principle from the 75% EtOH extract of freshly harvested seeds of *Cnestis palala* (LOUR.) MERR. (*C. ramiflora* GRIFF.) in a yield of 0.9%/fresh wt.

C. palala (Connaraceae) is a climbing shrub, growing in southeast Asia and the Malayan islands, which is laden with a velvety scarlet-orange fruit. A decoction of the roots of *C. palala* is traditionally prescribed to treat stomach-ache, malaria and urinary trouble.[4] Alternatively, the seeds are reported to be poisonous[5] and some Malays and Thais use the seeds (not fruits) for poisoning dogs.

This paper presents the structure and absolute configuration of a toxic principle (**1**) in the seeds of *C. palala*, and the distribution of this unusual amino acid in several parts of this plant. Acute toxicity of the seeds in a beagle dog is also described.

Results and Discussion

An aliquot of the 75% EtOH extract of the air-dried seeds, collected in Thailand, was subjected to Dowex 50W (H^+ form) ion-exchange column chromatography (CC) to separate the **1**-rich fraction by elution with 0.5 N NH_4OH solution. The separation of **1** from the **1**-rich fraction was achieved by the same CC (NH_4^+ form equilibrated with 0.1 N ammonium formate) by eluting with 0.1 N ammonium formate followed by a desalting procedure in the usual way. The isolated compound (**1**) shows a positive ninhydrin reaction (reddish-violet) on silica gel TLC. Compound **1** was assumed to be an amino acid from its profile on an automatic amino acid analyzer.

Compound **1** is a colorless, amorphous solid, $[\alpha]_D$ +35.0° (c=0.2, 1 N HCl). It has no absorption maximum above 220 nm in the UV spectrum. The IR spectrum of **1** indicated the presence of COO^- in the molecule. Positive ion FAB-MS showed the $[M+H]^+$ peak at m/z 181, indicating the molecular weight of 180 for **1**. Peaks of two methylene [27.1 (C-3) and 54.9 (C-4) ppm], a methine [55.9 ppm (C-2)], a carbonyl [175.8 ppm (C-1)] and a methyl [44.0 ppm (S-Me)] groups were observed in the ^{13}C-NMR spectrum of **1**, revealing it to be a derivative of methionine. In the ^1H-NMR spectrum, signals of a triplet [3.90 ppm (1H, J=6.1, 6.1 Hz, H-2)], two multiplets [3.47 and 2.39 ppm (each 2H, H-3 and 4] and a singlet [3.17 ppm (3H, S-Me)] were recorded, in accordance with the above suggestion. Downfield shifts of the S-Me carbon and hydrogens in the NMR spectra can be explained by the presence of an electron-withdrawing group on the sulfur atom. From the molecular weight, which is 31 mass units larger than that of methionine, the substituent was easily deduced to be sulfoximine (HN=S=O). In fact, authentic L-methionine S(*RS*)-sulfoximine migrated identically with **1** on silica gel TLC [*Rf* values, 0.18 (*n*-BuOH : AcOH : H_2O = 50 : 20 : 30/vol.); 0.38 (*n*-PrOH : H_2O = 60 : 40/vol.)][6] and also had the same retention time, eluting between tyrosine and phenylalanine, on an automatic amino acid analyzer (Hitachi 835-10).

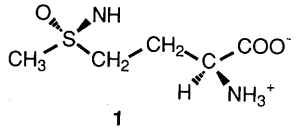

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Fig. 1

From these results, the structure of **1** was assumed to be methionine sulfoximine. The physico-chemical data (NMR and $[\alpha]_D$) also show that the natural methionine sulfoximine (**1**) is present as an optically pure form. Identification of the absolute configuration of the sulfur function of **1** was achieved by optical resolution of authentic L-methionine S(*RS*)-sulfoximine as reported by Rowe and Meister[7] and by Christensen and Kjae.[8] The absolute configuration of the resolved sulfoximine forming the more insoluble salt with (+)-camphor-10-sulfonic acid has been determined as 2(*S*), S(*R*) by X-ray analysis.[8] Although the two diastereomers (L-methionine S(*R*)-sulfoximine and L-methionine S(*S*)-sulfoximine) have closely similar specific rotation values,[9] the IR spectra of these compounds showed different peaks in the range of 1460—650 cm^{-1}. Therefore, the natural sulfoximine (**1**) was characterized by comparison with the two resolved diastereomers: the IR spectra of **1** was superimposable with that of the 2(*S*), S(*S*)-form (data are given in Experimental).

From the above results, it can be concluded that the absolute configuration of **1** is 2(*S*)-methionine S(*S*)-sulfoximine [(2*S*,S*S*)-2-amino-4-(S-methylsulfonimidoyl)-

n-butanoic acid].

Methionine sulfoximine was originally discovered to be the toxic factor in "agenised" flour producing hysteria in dogs,[10] and evidence for the presence of methionine sulfoximine in species of Connaraceae (*C. glabra*, *C. polyphyilla*, and *Rourea orientalis*) has been reported by Jeannoda *et al.*,[11] though they did not determine the absolute configuration. Therefore, this study is not only the first to describe the presence of **1** in *C. palala*, but also the first to report chemical determination of the absolute configuration of **1** isolated from a natural source.

Acute toxicity of the seeds of *C. palala* in a beagle dog was studied by the usual methods of toxicology.[12,13] A single oral administration of the pulverized seeds to a beagle dog at the dose level of 347 mg/kg produced no significant early toxicological findings except emesis, but after 15—18 h the animal showed serious clinical symptoms, such as emesis, lateral position, decreased locomotor activity, clonic and tonic convulsion, salivation, struggle and hyperpnea, and it died after 24—25 h. While some hematologic and blood biochemical parameters such as glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were increased 1 d after the administration, respiratory disorder appeared to be the cause of the death.

The amino acid composition and content in the EtOH extracts of several parts of *C. palala* have also been examined using an automatic amino acid analyzer. An extremely high concentration of **1** has been found in the seeds (524 μmol/g dry wt.), compared with other amino acids (each 0.3—20 μmol/g dry wt.). In addition, the presence of **1** was detected in the pods, petioles, roots, and stems (2.93, 9.92, 13.5, and 24.3 μmol/g dry wt., respectively). However, **1** was not detected in leaves collected in December. L-Methionine itself, considered to be the biosynthetic precursor of **1**, was not found in any of the parts examined. The questions of whether L-methionine is the precursor for the biosynthesis of **1**, and what is the physiological role of **1** in the intact plants, are under investigation.

Experimental

The instruments used to obtain physico-chemical data and the experimental conditions for chromatography were the same as those reported previously.[1-3]

Extraction and Isolation of 1 Plant materials of *C. palala* were collected in northern Thailand in December, 1991. The seeds (18.5 g) were ground in an agate mortar and pestle with 75% EtOH and a spoonful of sea sand. The solution was centrifuged (3000 rpm, 15 min), and the supernatant was evaporated to dryness below 45 °C to afford the EtOH extract. This EtOH extract (2.8 g) was dissolved in H_2O (15 ml) and filtered. The filtrate was evaporated to dryness to give a residue (2.6 g). This was chromatographed on a Dowex 50W × 4 column (H^+ form, 100—200 mesh, 4.0 × 48 cm) equilibrated with distilled H_2O. The amino acid fraction (0.5 g) was eluted with 0.5 N ammonia solution after washing with distilled H_2O (2 l). This fraction was dissolved in 0.1 N ammonium formate buffer (pH 3.25) and the solution was applied to a Dowex column (NH_4^+ form, 4.0 × 48 cm) equilibrated with 0.1 N ammonium formate buffer (pH 3.25). The column was first washed with this buffer (4 l), and then eluted with 0.1 N ammonium formate buffer (pH 4.25). Each fraction (150 ml) was collected and monitored by TLC (*n*-BuOH : AcOH : H_2O = 5 : 2 : 3, v/v). Compound **1**-rich fractions were combined and evaporated to dryness. The residue was next chromatographed on the same Dowex column as above to remove the buffer salts by washing with distilled H_2O (2 l), and then eluted with 1 N ammonia solution. The ammonia was eliminated by evaporation to dryness to give crude **1** (119 mg). This was washed with a mixture of EtOH–MeOH (20 + 1 ml). The alcohol-soluble part was decanted and evaporated to give a white residue. This procedure was repeated twice more to give pure **1** (92 mg).

Compound 1 Colorless amorphous solid, $[\alpha]_D$ +35.0° (c = 0.2, 1 N HCl). UV: end absorption (H_2O). IR ν_{max}^{KBr} cm^{-1}: 3500—2500, 2150, 1640, 1590, 1540, 1440, 1400, 1370, 1340 (sh), 1320, 1280, 1260 (sh), 1200, 1160, 1110, 1070, 1020, 1000 (sh), 960, 950, 860, 820, 770, 750, 700, 660. Positive ion FAB-MS (glycerol) m/z: 181 $[M+H]^+$, 167, 165, 115, 102. ^{1}H-NMR (D_2O, 500 MHz) δ ppm from tetramethylsilane (TMS): 3.90 (1H, t, J = 6.1, 6.1 Hz, H-2), 3.47 (2H, m, H-4), 2.39 (2H, m, H-3), 3.17 (3H, s, S-Me). ^{13}C-NMR (D_2O, 125 MHz): δ ppm from TMS: 27.1 (CH_2, C-3), 44.0 (CH_3, S-Me), 54.9 (CH_2, C-4), 55.9 (CH, C-2), 175.8 (C=O, C-1).

Optical Resolution of L-Methionine S(*RS*)-Sulfoximine[7,8] (+)-Camphor-10-sulfonic acid (2.43 g, 10 mmol) and L-methionine S(*RS*)-sulfoximine (0.90 g, 5 mmol) were dissolved in hot *n*-propanol (25 ml). The solution was filtered and the filtrate was kept at room temperature overnight. Precipitates formed were collected by filtration and the resultant solution was allowed to stand at 5 °C for 2 d. The crystals formed were collected by filtration and recrystallized repeatedly from EtOH–AcOEt mixture at 5 °C to give the camphor sulfonate salt of L-methionine S(*R*)-sulfoximine (**1a**, 260 mg). The filtrate was evaporated to dryness and the residue was recrystallized from EtOH–AcOEt mixture in the same manner as above to remove remaining **1a**. The final solution was evaporated to dryness to afford the camphor sulfonate salt of L-methionine S(*S*)-sulfoximine (**1b**, 1.0 g). Compounds **1b** and **1a** were separately chromatographed on Dowex 50W × 4 (H^+ form) with 0.5 N ammonia solution to give **1** (72 mg) and a fraction rich in its diastereomer (**2**) (406 mg), respectively. The latter was repeatedly washed with EtOH–MeOH mixture to afford pure **2** (293 mg). **1**: IR ν_{max}^{KBr} cm^{-1}: 3500—2500, 2150, 1640, 1590, 1440, 1400, 1370, 1340 (sh), 1320, 1280, 1260, 1210 (sh), 1200, 1160 (sh), 1110, 1070, 1020, 1000 (sh), 960, 950 (sh), 910, 860, 840, 820, 770, 750, 700, 660. **2**: IR ν_{max}^{KBr} cm^{-1}: 3500—2500, 2100, 1640, 1590, 1540, 1440, 1400, 1340, 1320 (sh), 1310, 1280, 1260, 1210, 1160, 1110 (sh), 1070, 1020, 950, 910, 840, 770, 720, 700, 660.

Acute Toxicity of the Seeds of *C. palala* in a Beagle Dog[12,13] Two male beagle dogs, 18 months old, were used in this study. The pulverized seeds were administered orally at the dose level of 347 mg/kg in a gelatin capsule. The control dog received gelatin capsules only. The animals were examined during 0—7 h and at 1 d after the treatment for mortality and gross signs of toxicologic and pharmacologic effects. Hematologic and blood biochemical determinations were performed at 2, 4, 6 h and 1 d after the treatment, on blood samples taken from the cephalic vein.

Amino Acid Composition and Content in Several Parts of *C. palala* EtOH extracts of pods, petioles, roots, leaves, and stems were prepared in the same manner as described above. Amino acid analyses of these samples were performed with a Hitachi 835-10 automatic amino acid analyzer using

Table I. Amino Acid Composition and Content in Several Parts of *Cnestis palala* (μmol/g dry wt.)

Amino acid	Seeds	Pods	Leaves	Petioles	Roots	Stems
Asp	4.50	1.64	0.40	0.16	1.53	0.39
Thr	1.04	0.39	0.47	0.18	0.28	0.25
Ser	3.71	1.50	0.66	0.86	0.73	1.00
Asn	21.3	108	2.10	5.92	46.0	14.9
Glu	10.2	1.72	0.54	0.11	0.79	0.10
Gln	5.24	7.06	2.35	0.85	2.36	0.43
Pro	3.10	4.15	0.88	0.33	0.18	0.35
Gly	1.21	0.45	0.36	0.08	0.25	0.13
Ala	8.03	2.95	7.32	1.55	1.96	2.18
Val	1.67	0.51	0.42	0.21	0.22	0.36
Cys	0.54	0.67	0.75	0.09	0.10	0.18
Met	0	0	0	0	0	0
Ile	0.64	0.08	0.21	0.13	0.11	0.18
Leu	0.30	0.11	0.27	0.11	0.08	0.16
Tyr	0.94	1.02	0	0.11	0.10	0.08
Phe	0.50	1.24	0.10	0.11	0.12	0.08
MSO[a]	524	2.93	0	9.92	13.5	24.3
GABA	11.2	5.99	4.49	1.38	1.46	1.66
Orn	0.33	0	0.06	0	0	0
Lys	0.73	0.99	0.14	0.03	0.04	0.06
His	0.54	0	0.04	0.03	0.09	0.04
Arg	15.2	1.71	0.18	0.08	0.70	0.52

a) MSO: Methionine sulfoximine.

the Li–citrate buffer system as reported previously (Table I).[14)]

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