# The Isolation and Structure Elucidation of a New Sesquiterpene Lactone from the Poisonous Plant *Coriaria japonica* (Coriariaceae)

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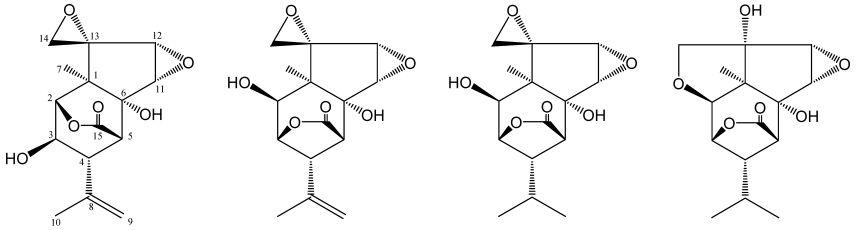
**A new sesquiterpene lactone named coriarin was isolated from achenes (seeds) of *Coriaria japonica* (Coriariaceae) along with known constituents tutin, dihydrotutin and corianin, and its structure was deduced on a spectroscopic basis. The structure of coriarin was finally confirmed by the base-catalized chemical conversion of tutin into coriarin, in which the lactone ring linkage was transposed from C-3 to C-2. Chemical investigation of sarcocarps was also undertaken in parallel, but neither sesquiterpene lactones nor related constituents were obtained. The results indicate that sesquiterpene lactones occur only in achenes of *C. japonica* berry, as is the case in other *Coriaria* species.**

**Key words** *Coriaria japonica*; Coriariaceae; sesquiterpene lactone; poisonous plant

Coriariaceae is a monotypic family consisting of about sixteen species, which occur in the Mediterranean region, the East Asia region extending from the Himalayas and China through Japan, Taiwan and Philippines, the southern hemisphere region from Papua-New Guinea and New Zealand to the Andes, and the Central America-Northern South American region. *Coriaria japonica* is a small deciduous shrub endemic to Japan, and grows mainly in barren areas such as river beds and volcanic fields in Hokkaido and Honshu Island east of the Kinki region. All parts of this plant are known to contain toxic sesquiterpene lactones that are fatal to mammals in small doses, and there have been occasional incidences of poisoning due to the ingestion of mature berries. It is usually infants that fall victim to the poisoning of this plant since they are attracted to the sweet black berries. The berry of *C. japonica* as well as the rest of *Coriaria* species is a pseudocarp consisting of sarcocarp, which is developed morphologically from the calyx, and many small achenes, which are true fruits and enfolded by sarcocarps. It is said that the sarcocarp of some *Coriaria* species tastes sweet and is thus edible, whereas the toxic principles are confined to achenes.[1)] In Europe, the sap juice prepared from *C. myrtifolia* berry has been drunk raw or as wine after fermentation. In the Himalayan area, *Coriaria* berry is considered to be edible unless the seeds (achenes) are ingested in a crushed form. However, it is uncertain whether that is the case with *C. japonica* or not, since the ingestion of its berry has been thought to be very dangerous due to poisoning incidences in Japan. Quite recently, the isolation of an unprecedented sesquiterpene basic skeleton, named coriane, along with a novel highly oxygenated picrotoxane-type sesquiterpene from *C. nepalensis* was reported.[2)] Chemical investigations on most *Coriaria* species were undertaken decades ago.[3—6)] These circumstances inspired us to undertake detailed chemical investigations on both sarcocarps and achenes separated from berries of *C. japonica* in a search for new types of sesquiterpene lactones. This paper refers to the isolation and structure elucidation of a new sesquiterpene lactone from *Coriaria japonica* berries.

The acetone extracts of both achenes and sarcocarps separated from *C. japonica* berries were subjected to a series of column chromatography over silica gel and reversed-phase silica gel. The achene extract yielded a new compound, coriarin (**1**), and three known compounds, tutin (**2**),[7)] dihydrotutin (**3**)[8)] and corianin (**4**).[7)] Though the isolation of dihydrotutin (**3**) has been reported from *Picrodendron baccatum*,[8)] there has been no solid scientific evidence for its occurrence in *Coriaria* species. As for the sarcocarp extract, neither sesquiterpene lactones nor related constituents were obtained from this extract, and thus chemical studies on this part were not pursued further.[9)]

Coriarin (**1**) was isolated as optically active prisms, mp 203—204 °C, $[\alpha]_D^{22}$ −34.5 °. The molecular formula of **1** was deduced as $C_{15}H_{19}O_6$ by high-resolution mass spectroscopy. The IR spectrum showed an absorption band assignable to the lactone at 1756 $cm^{-1}$. The structure for **1** was most effectively elucidated by a combination of 1D- and 2D-NMR spectroscopy as mentioned below. The $^1$H-NMR spectrum exhibited resonances for one tertiary methyl at $\delta$ 1.37 (3H, s, 7-$CH_3$) and one allyl methyl at $\delta$ 2.09 (3H, s, 10-$CH_3$) along



**1** **2** **3** **4**

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with olefinic protons typical of an *exo*-methylene group at $\delta$ 5.10 and 5.41 (1H each, s, 9-$H_2$). The presence of the epoxide was evident from the resonances for two vicinal oxymethine protons at $\delta$ 3.63 (1H, d, $J$=2.6 Hz, 12-H) and $\delta$ 4.09 (1H, d, $J$=2.6 Hz, 11-H), which were correlated with secondary oxygenated carbon signals at $\delta$ 60.6 (12-C) and 63.1 (11-C), respectively, in the HMQC spectrum. There was another pair of proton signals at $\delta$ 3.23 (1H, d, $J$=4.2 Hz) and $\delta$ 3.39 (1H, d, $J$=4.2 Hz), which the HMQC spectrum indicated were directly attached to an oxygenated carbon of $\delta$ 52.4. A small coupling constant (4.2 Hz) for geminal protons was indicative of the oxymethylene group occurring as a terminal epoxide. The $^1$H-NMR spectrum also indicated the presence of a chain of four methine protons in the range $\delta$ 3.10—4.96, which were confirmed by the $^1$H–$^1$H COSY. A proton signal at $\delta$ 4.96 was observed as a broad double doublet signal and shared a coupling constant of 6.2 Hz with a signal at $\delta$ 3.10, 6.3 Hz with a signal at $\delta$ 3.10 that was assigned to 3-OH, and a very small coupling constant with a signal at $\delta$ 4.73. A small coupling constant was also shared between signals at $\delta$ 3.76 and 3.10. The above spectral analysis readily assigned a chain of methines as ■–CH(O–■)–CH(OH)–CH–CH–■. The connectivities between those partial structures deduced above were finally determined by HMBC correlations as illustrated in Fig. 1. The stereochemical assignments were based on the results of the selected NOESY spectrum as illustrated in Fig. 2. Small coupling constants between some proton signals in a chain of methines were also explained from the stereochemical aspects revealed by NOESY data. The above spectroscopic findings established the structure of coriarin as **1**.[10] Coriarin is an isomeric form of tutin (**2**) in which the lactone ring linkage is transposed from C-3 in tutin (**2**) to C-2 in coriarin (**1**). One of the two oxymethylene protons at C-14 in tutin (**2**) is known to exhibit a marked downfield shift by more than 1 ppm as compared to the corresponding proton signal of coriamyrtin, which is ascribed to the deshielding effect of 2-OH that is in close proximity to 14-H.[7] In coriarin (**1**), such proximity of 14-H to oxygen functionality at C-2 was lost since it is incorporated into a rigid lactone ring. Since coriarin (**1**) was obtained in small quantity, preparation of this metabolite from the main constituent tutin (**2**) was attempted (Fig. 3). Since the core structure of coriarin (**1**), "2-oxabicyclo[2.2.2]octan-3-one", is considered to be more stable than that of tutin (**2**), that is, "6-oxabicyclo[3.2.1]octan-7-one", we considered it possible to convert tutin (**2**) into coriarin (**1**). First, a solution of **2** in toluene was heated in a sealed tube in an attempt to convert **2** into **1** thermodynamically, but this attempt failed. Second, we tried the base-catalyzed conversion of tutin (**2**) into coriarin (**1**) on treatment with sodium hydride in dry tetrahydrofuran, and this conversion was successfully accomplished in the yield of 19%.

The chemistry of *C. japonica* was initiated practically by Okuda *et al.* in the 1950s,[3,4] and sesquiterpene lactones isolated from this species were coriamyrtin, tutin (**2**) and corianin (**4**). Our research added dihydrotutin (**3**) and a new compound coriarin (**1**) to the list of constituents isolated from this species, but could not obtain coriamyrtin, which is considered to be a main constituent of *C. japonica*. The genus *Coriaria* is distributed, as stated above, in four major separate areas in the world, which is one of the most conspicuously disjunct distributions in the kingdom of flowering plants. Though the areas of distribution of the genus *Coriaria* are geographically isolated from one to another and thus each species or group of species is expected to have undergone a high level of differentiation since its origination, the chemical spectrum of each species is very similar with either coriamyrtin or tutin as a main constituent. Only *C. nepalensis* is known to yield metabolites distinct from ordinary sesquiterpene lactones such as coriamyrtin and so on.[2] The recent molecular phylogenic study on *Coriaria* species suggested that those occurring in the regions from Papua-New Guinea and New Zealand through the Pacific Islands and Chile, and the Central America-Northern South American species form a sister group to which the Eurasian clade is basal.[11] This seems to indicate that the genus *Coriaria*, which also accounts for the monotypic family Coriariaceae,

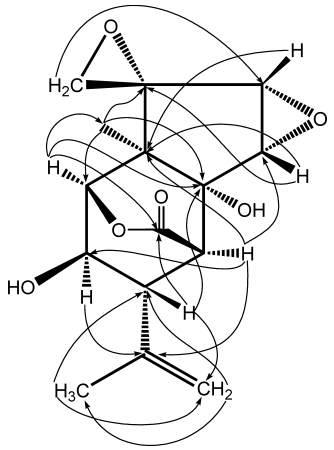


Fig. 1. HMBC Correlations Observed in Coriarin (**1**)

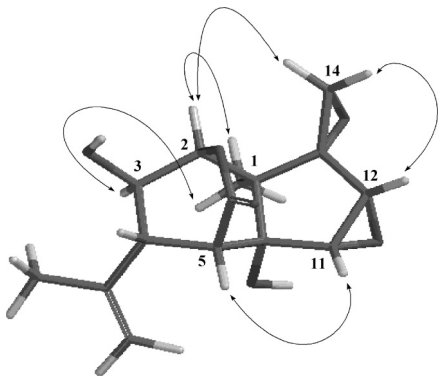


Fig. 2. Molecular Model of Coriarin (**1**) with Arrows Representing Main NOESY Correlations

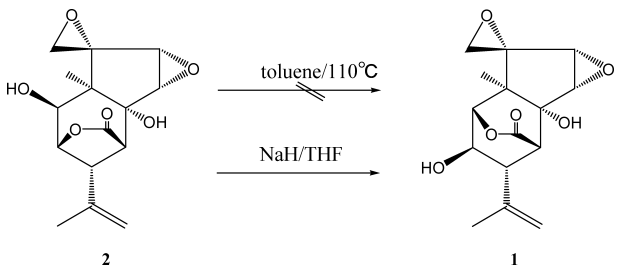


Fig. 3. Chemical Conversion of Tutin (**2**) into Coriarin (**1**)

originated from the Himalayan region. This may explain why the Himalayan species is the most differentiated from a chemosystematic viewpoint, thus yielding characteristic sesquiterpene lactones.[2)]

Picrotoxanes are structurally in close relation to *Coriaia* lactones in that both are highly oxygenated terpenes. It is interesting to note that both types are known to act as antagonists of ionotropic GABA receptors and some structure–activity relationships were also described.[12)] Since coriarin has a partial structure of 2-oxabicyclo[2.2.2]octan-3-one distinct from other *Coriaria* lactones and picrotoxanes, it may exhibit a different type of pharmacological activity.

**Experimental**

All melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. Spectral data were obtained using the following apparatus: $^{1}$H- and $^{13}$C-NMR spectra with a Bruker DRX-500 ($^{1}$H, 500 MHz; $^{13}$C, 125 MHz) spectrometer at 300 K in $C_5D_5N$. The chemical shift ($\delta$) of proton signals is given in ppm relative to the resonance of residual $C_5D_4\underline{H}N$ at 7.21 ppm and that of carbon signals is given in ppm relative to the resonance at 135.5 ppm for $C_5D_5N$; mass spectra (MS) and high-resolution mass spectra (HR-MS) with a VG AutoSpec E and Micromass LCT (Manchester, U.K.) mass spectrometer; IR spectra with a JASCO FT/IR 620 infrared spectrometer; optical rotations with a JASCO DIP-360 polarimeter. Column chromatography was carried out with Wakogel C-200 or Merck Kieselgel 60 (eluted with hexane–acetone). Preparative HPLC was carried out on a JASCO PU-880 equipped with a UV-970 UV detector (230 nm) and a Phenomenex Luna ODS column (15 $\mu$m, 21.2 mm×250 mm) on elution with $H_2O$–MeOH or $H_2O$–$CH_3CN$ at a flow rate of 8 ml/min. Thin-layer chromatography (TLC) was conducted on 0.25 mm pre-coated silica-gel plates ($60F_{254}$, Merck), and spots were detected by inspection under short (254 nm) or long (365 nm) wavelength UV lights, or by the colors developed with 10% $H_2SO_4$ spraying followed by heating on a hot plate.

**Plant Material** Matured berries (11.87 kg) of *C. japonica* were collected from the corresponding plants cultivated at the Medicinal Plant Garden, Teikyo University, Japan, in June 2000, and were separated into achenes (2.07 kg) and sarcocarps (9.8 kg). A voucher specimen in complete form with flowers is on deposit at the Herbarium of Medicinal Plant Garden, Teikyo University.

**Extraction and Isolation** Both achenes and sarcocarps of *C. japonica* were dried, crushed and extracted with acetone three times at room temperature. The acetone extract (700 g) of achenes was suspended in aq. MeOH (1400 ml), and percolated with benzene three times (400 ml each). The aq. MeOH layer was evaporated *in vacuo* to furnish an oily residue (12.6 g). The residue was then chromatographed over silica gel, and the column was eluted with the following solvent system: (1) hexane; (2) hexane–acetone (4 : 1); (3) hexane–acetone (2 : 1); and (4) methanol. Chromatographic fractions collected from the above column were combined into five fractions as follows: fr. 1 (3.70 g); fr. 2 (0.35 g); fr. 3 (0.69 g); fr. 4 (1.02 g); fr. 5 (2.86 g). Fraction 3 was recrystallized from hexane–dimethylether to give the crude crystalline mixture (185 mg), which was then chromatographed over ODS on elution with $H_2O$–$CH_3CN$ (4 : 1) to give nine chromatographic fractions, fr. 3C-1—fr. 3C-9. Recrystallization of fr. 3C-2, fr. 3C-3 and fr. 3C-4 afforded tutin[7)] (**2**; 154 mg), corianin[7)] (**4**; 9.0 mg) and dihydrotutin[8)] (**3**; 0.73 mg), respectively, in pure forms, which were identified with authentic samples by comparison with their spectral data reported in the literature. The mother liquor was evaporated to dryness, and the residue (502 mg) was also subjected to chromatographic separation over ODS on elution with $H_2O$–$CH_3CN$ (4 : 1) and fractionated into fifteen fractions, fr. 3M-1—fr. 3M-15. Recrystallization of fr. 3M-3 and fr. 3M-6 afforded tutin (**2**; 19.4 mg) and corianin (**4**; 13.6 mg). Fraction 3C-8 and fr. 3M-2 were combined and purified by HPLC over ODS on elution with $H_2O$–$CH_3CN$ (1 : 9) to give coriarin (**1**; 3.2 mg) in pure form. The acetone extract of sarcocarps (1.3 kg) was also processed in the same manner, but sesquiterpene lactones and their relatives were not observed.

**Coriarin (1)** Colorless prisms from MeOH, mp 203—204 °C. $[\alpha]_D^{22}$ −34.5° ($c$=0.116, MeOH). IR (film): 3443 (OH), 1756 (C=O). $^{1}$H-NMR (500 MHz, pyridine-$d_5$, 300 K) $\delta$: 1.37 (3H, s, 7-$CH_3$), 2.09 (3H, s, 10-$CH_3$), 3.10 (1H, br d, $J$=5.1 Hz, 4-H), 3.23 (1H, d, $J$=4.2 Hz, 14-H), 3.39 (1H, d, $J$=4.2 Hz, 14-H), 3.63 (1H, d, $J$=2.6 Hz, 12-H), 3.76 (1H, d, $J$=1.5 Hz, 5-H), 4.09 (1H, d, $J$=2.6 Hz, 11-H), 4.73 (1H, s, 2-H), 4.96 (1H, dd, $J$=6.2, 6.3 Hz, 3-H), 5.10, 5.41 (1H each, s, 9-$H_2$), 7.40 (1H, d, $J$=6.3 Hz, 3-OH), 7.84 (1H, s, 6-OH). $^{13}$C-NMR (125 MHz, pyridine-$d_5$, 300 K) $\delta$: 15.7 (7-C), 21.7 (10-C), 46.6 (1-C), 50.2 (4-C), 50.4 (5-C), 51.6 (14-C), 60.6 (12-C), 63.1 (11-C), 66.3 (13-C), 67.1 (3-C), 79.0 (6-C), 87.7 (2-C), 110.2 (9-C), 144.6 (8-C), 174.1 (15-C). HI-ESI-MS: $m/z$ 295.1194 (Calcd for $C_{15}H_{19}O_6$: 295.1182).

**Chemical Conversion from Tutin (2) into Coriarin (1)** To a tetrahydrofuran solution (4 ml) of tutin (**2**; 24 mg, 0.0082 mmol) was added sodium hydride (60%, 8.2 mg, 0.205 mmol) at room temperature. The reaction mixture was stirred at the same temperature for 15 h. Saturated ammonium chloride solution (10 ml) was added to the reaction mixture, which was then extracted with ethyl acetate (10 ml×2). The combined organic layer was dried over anhydrous magnetic sulfate, and evaporated *in vacuo* to give a solid, which was purified with HPLC ($H_2O$–$CH_3CN$ 9 : 1) to give coriarin (**1**) as a colorless solid (4.6 mg, 19% yield).

**References and Notes**


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9) The finding that the sarcocarp extract contains no toxic principles was also supported by a toxicity experiment using mice (T. Kinoshita, unpublished data). The sarcocarp extract consisted predominantly of polyphenols, as is the case for other ordinary black berries. We did not proceed further since it had nothing to do with the goal of this research.
10) A search of the literature in the CAS database for the following structural name (spiro[indan-1,2′-oxirane]-4-carboxylic acid, 2,3-epoxyhexahydro-3a,6,7-trihydroxy-5-isopropenyl-7a-methyl-, $\gamma$-lactone) led to us finding the following literature: Okuda T., Yoshida T., *Tetrahedron Lett.*, **1965**, 4191—4197 (1965). However, there was no description concerning the corresponding compound in this paper. In the subsequent full paper of this article by the same authors, a compound structurally corresponding to a dihydro form of coriarin, that is, spiro[indan-1,2′-oxirane]-4-carboxylic acid, 2,3-epoxyhexahydro-3a,6,7-trihydroxy-5-isopropyl-7a-methyl-, $\gamma$-lactone, was described as an intemediate compound in the course of chemical correlation between coriamyrtin and tutin. Perhaps, what was found as spiro[indan-1,2′-oxirane]-4-carboxylic acid, 2,3-epoxyhexahydro-3a,6,7-trihydroxy-5-isopropenyl-7a-methyl-, $\gamma$-lactone in the CAS database will be counted as one of the possible candidates for the structure of tutin and thus has no corporeality. Therefore, coriarin (**1**) is a new compound from not only natural sources but also artificial products.
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