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CHEMICAL COMPOSITION OF *Capparis spinosa* FRUIT

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The known compounds cappariloside A and stachydrin, an adenosine nucleoside, and for the first time from plants of the Capparidaceae family the known compounds hypoxanthine and uracil were isolated from Capparis spinosa *(Capparidaceae) fruit.*

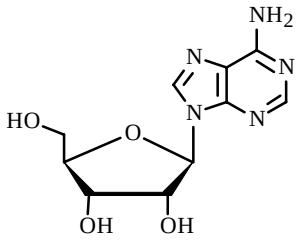
Key words: *Capparis spinosa* L., cappariloside A, stachydrin, purine base, adenosine, hypoxanthine, uracil.

Capparis spinosa (Capparidaceae) is widely distributed throughout the whole world. Information on alkaloids, flavonoids, and glycosides [1-5] in addition to lipids and carbohydrates [6] from this plant has been published.

Herein we report a study of alkaloids from fruit of *C. spinosa* growing in Xinjiang Autonomy Region of China.

The alcohol extract of ground and defatted ripe fruit of *C. spinosa* produced total extracted substances containing also water-soluble alkaloids of the betaine type. The lipophilic components were removed by washing the acidic solution of extracted substances with ether. The total alkaloids were obtained by treatment of the acidic solution with conc. ammonia to adjust the pH to 9 and extraction with *n*-butanol (fraction A). The dried alkaloidal fraction A was chromatographed over a silica-gel column with elution by $CHCl_3$, $CHCl_3$:CH_3OH, and CH_3OH. Work up with CH_3OH of the $CHCl_3$:CH_3OH (12:1) fractions isolated amorphous hypoxanthine (**1**) and uracil (**2**) [7, 8], which were identified using PMR and ^{13}C NMR spectral data and authentic samples (spectral properties are given in Experimental).

Crystalline **3**, mp 228-229°C, was isolated from $CHCl_3$:CH_3OH (10:1) fractions. The UV spectrum of **3** had absorption maxima at 206.4 and 259.6 nm. The IR spectrum had absorption bands for active H at 3425, 3370 (NH_2), 3320, and 3143 (OH) cm^{-1}; lactone ring (tetrahydrofuran), 1680; ether, 1100 and 1030; and tri- and disubstituted aromatic rings, 1600, 1577, 870, 822, 795, and 765. The mass spectrum of **3** gave a peak for the molecular ion with *m*/*z* 267 and fragments with *m*/*z* 148 and 119 produced by cleavage of the tetrahydrofuran ring. Peaks for ions with *m*/*z* 134 and 133 corresponded to fragments formed by cleavage of the C–N bond between the main part of the molecule and the tetrahydrofuran ring. NMR data (^1H and ^{13}C) are given in Experimental.



3

The spectral data (UV, IR, mass, NMR) were reminiscent of those of adenosine (**3**) [9]. However, the lack of an authentic sample prohibited reliable identification of **3** as adenosine. As a result, a single-crystal x-ray structure analysis (XSA) of **3** showed that the isolated base was in fact the known nucleoside adenosine ($C_{10}H_{13}N_5O_4$), which is constructed from D-ribose and a purine base in which the N-9 atom of the purine base adenine is bonded to C-1 of D-ribose [10-13]. Adenosine

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is widely distributed in the animal and plant world and is found in the free state in certain plants (tea, sugar beets, hops, mushrooms), in most yeast and bacteria, in animal organs (muscle, liver, corpus luteum), and in urine. The bound form occurs in nucleic acids and enzymes.

Continued elution of the column with $CHCl_3$:CH_3OH (8:1) isolated from separate fractions an amorphous compound $C_{16}H_{18}N_2O_6$, which was identified as the known compound cappariloside A (**4**) [14] based on spectral data (UV, IR, mass, PMR, ^{13}C NMR).

Alkaloids were precipitated as the complex with $NH_4[Cr(NH_3)_2(CNS)_4]\cdot H_2O$ after work up of the alkaline solution with *n*-butanol and acidification of the aqueous residue with dilute mineral acid. The complex was decomposed over an Al_2O_3 column to isolate a compound that was identified by mixed melting point and spectral data (UV, IR, mass, PMR) as the known alkaloid stachydrin (**5**) [15].

EXPERIMENTAL

General Comments. UV spectra were recorded on a UV-2550 UV—Visible spectrophotometer (Shimadzu); IR spectra (in KBr disks), on a Nicolet FTIR-Magna 750 spectrophotometer. NMR spectra in DMSO-d_6 were recorded on a Varian Inova spectrometer (400 MHz for ^1H; 100 MHz, ^{13}C). Mass spectra were obtained in a MAF-95 double focusing spectrometer. Melting points were determined on a Fisher—Johns apparatus. Analytically pure solvents were used in all experiments.

Plant Material. Ripe fruit of *C. spinosa* were collected in Xinjiang province in June 2004. Samples were identified in Xinjiang Institute of Ecology and Geography of the Chinese Academy of Sciences.

Extraction and Isolation. Ground plant material (5 kg) was soaked at room temperature in ethanol (70%) for 1 d. The ethanol extract was decanted daily. A total of four decantations was made. These were combined and condensed in vacuo. The concentrated extract was diluted with water, acidified with H_2SO_4 solution (5%), and washed three times with ether. The acidic aqueous solution was made basic with conc. NH_3 solution until the pH was 10 and worked up with *n*-butanol. The butanol extracts were dried over calcined potash, filtered, and condensed. The residue was dried in vacuo to produce the alkaloid fraction (52 g), which was chromatographed over a silica-gel column. Alkaloids were eluted with $CHCl_3$, $CHCl_3$:CH_3OH, and CH_3OH. The $CHCl_3$:CH_3OH fractions (12:1) were worked up with CH_3OH to produce amorphous hypoxanthine (**1**) and uracil (**2**). PMR and ^{13}C NMR spectral data of **1**: PMR (ppm): 8.115 (1H, s, H-8), 7.975 (1H, s, H-2); ^{13}C NMR: 155.590 (C-6), 153.322 (C-4), 144.922 (C-2), 140.365 (C-8), 119.251 (C-5); **2**: PMR (J/Hz): 7.400 (1H, d, J = 8.0, H-6), 5.461 (1H, d, J = 8.0, H-5); ^{13}C NMR: 164.163 (C-4), 151.256 (C-2), 141.989 (C-6), 100.119 (C-5).

The $CHCl_3$:CH_3OH fraction (10:1) afforded an amorphous substance that was chromatographed again over a silica-gel column to produce adenosine (**3**), mp 228-229°C. PMR (J/Hz): 8.357 (1H, s, H-2), 8.141 (1H, s, H-8), 5.877 (1H, d, J = 6.4, H-1′), 4.607 (1H, t, J = 6.4, H-2′), 4.136 (1H, dd, J = 6.4, 3.6, H-3′), 3.968 (1H, q, J = 3.6, H-4′), 3.667 (1H, dd, J = 12.4, 3.6, H-5′), 3.545 (1H, dd, J = 12.4, 3.6, H-5″); ^{13}C NMR: 152.30 (C-2), 148.30 (C-4), 119.14 (C-5), 155.91 (C-6), 139.86 (C-8), 87.73 (C-1′), 73.18 (C-2′), 70.44 (C-3′), 85.71 (C-4′), 61.45 (C-5′).

The $CHCl_3$:CH_3OH fractions (8:1) afforded an amorphous compound $C_{16}H_{18}N_2O_6$, cappariloside A (**4**).

UV spectrum (MeOH): 267, 278, 289 nm. IR spectrum (cm^{-1}): 3525, 3495, 3400 (OH, NH); 2250 (CN); 1625, 1590, 1170, 1080 (C–O–C). Mass spectrum: 333 $[M - 1]^+$.

The basic aqueous solution was acidified with H_2SO_4 (5%) until the pH was 2, worked up with *n*-butanol, and treated with $NH_4[Cr(NH_3)_2(CNS)_4]\cdot H_2O$. The complex of total alkaloids that precipitated from the aqueous solution (fraction B) was separated and chromatographed over an Al_2O_3 (neutral) column with elution by $CHCl_3$:CH_3OH (4:1). Stachydrin (**5**), mp 235-236°C, was isolated from separate fractions.

X-ray Structure Analysis. Unit-cell constants of **1** were determined and refined on a Stoe Stadi-4 diffractometer (T = 300K, graphite monochromator). The principal crystallographic parameters for the structure of **1** were identical within experimental uncertainty to those published [9]. A three-dimensional set of reflections was collected on the same diffractometer by $\omega/2\theta$-scanning using Mo Kα-radiation. Absorption corrections were not applied. The structure of **1** was solved by direct methods using the SHELXS-97 programs. The structure was refined using the SHELXL-97 program. All nonhydrogen atoms were reinfed by anisotropic full-matrix least-squares methods (over F^2). Positions of H atoms were found geometrically and refined with fixed isotropic thermal parameters $U_{iso} = nU_{eq}$, where n = 1.5 for methyls and 1.2 for others and U_{eq} is the equivalent isotropic thermal parameter of the corresponding C atom. Hydroxyl H atoms were found in a difference electron-density synthesis.

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