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STEROIDS AND TRITERPENOIDS FROM *Cucumis sativus* ROOTS

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Investigations on the EtOH extract of Cucumis sativus *roots led to the isolation of 15 compounds (**1–15**). Their structures were identified using spectroscopic methods. Among these compounds, compound **1**, stigmasta-8(14),22-diene-7α-methoxy-3β-ol, is a new steroid, and **2**, **4–15** were isolated from this plant for the first time.*

Keywords: *Cucumis sativus*, Cucurbitaceae, steroids, triterpenoids.

Cucumber is well known as a nutritive vegetable. The roots of *Cucumis sativus* (Cucurbitaceae), with the Chinese name Huang-Gua-Gen, have been used as a traditional herb for the treatment of diarrhea and dysentery in China. A formula that includes this herb is also used to treat diabetes [1]. Previous reports indicated that the major principles of *C. sativus* are bitter compounds such as triterpenoids and steroids [2–4]. However, compounds in the roots of *C. sativus* are not known so far. This study led to the isolation of 15 compounds. Among them, compound **1** is a new steroid and **2**, **4–15** were isolated from this plant for the first time.

Compound **1** was obtained as a white amorphous solid. The molecular formula $C_{30}H_{50}O_2$ was derived from HR-ESI-MS, ^{13}C NMR, and DEPT spectra. The ^{13}C NMR spectrum showed 30 carbons, including six methyls, an oxygen-bearing methyl, nine methylenes, ten methines (two olefinic ones and two oxygen-bearing ones), and four quaternary carbons (two olefinic ones). The NMR data of **1** was indicative of a steroid similar to the aglycone of compound **3**. The main difference was at C-7, C-8, and C-14. ^1H, ^1H correlation of H-6/H-7 (δ 4.00), HMBC correlations of OCH_3 (δ 3.11)/C-7 (δ 74.8), H-7, H-9/C-8 (126.3), C-14 (149.3), and Me-18/C-14 indicated the presence of a $\Delta^{8,14}$ double bond and the position of OCH_3 (Fig. 1). The ROESY spectrum showed the correlation between H-3 (δ 3.50) and H-5 (δ 1.72), suggesting H-3 and H-5 were vicinally oriented. ROESY correlation of CH_3-18/CH_3-21 indicated that CH_3-18 and H-17 were at the opposite side. The scarcity of efficient ROESY correlations for H-7 or OCH_3 made it difficult to determine the relative configuration at C-7. The orientation of OCH_3 was assigned as the α-form by structure model study and from the J value of H-7. The small $J_{H\text{-}6,H\text{-}7}$ value (2.5 Hz) was attributed to the dihedral angles of Ha-C(6)–C(7)-7 and Hb-C(6)–C(7) being both ca. 60°; in this circumstance, only the α-oriented OCH_3 could avoid the large spatial hindrance between CH_3-19 and OCH_3. The geometry of $\Delta^{22,23}$ was of the *E*-form, as deduced from the large $J_{H\text{-}22,H\text{-}23}$ value of 15.1 Hz. The chemical shift of CH_3-21 was δ 1.08, corresponding to the 20*R*-configuration (20*S*: δ 1.28; 20*R*: δ 1.13) [5, 6]. The absolute configuration at C-24 has not been determined yet. Thus, the structure of **1** was deduced as stigmasta-8(14),22-diene-7α-methoxy-3β-ol.

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TABLE 1. ^{1}H and ^{13}C NMR Data of Compound **1** ($(CD_3)_2CO$, δ, ppm, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
1	37.7 (CH_2)	2.00 (m)	15a	26.1 (CH_2)	2.45 (m)
2a	32.3 (CH_2)	1.74 (m)	15b		2.29 (m)
2b		1.33 (m)	16a	28.6 (CH_2)	1.79 (m)
3	71.0 (CH)	3.50 (m)	16b		1.45 (m)
4a	38.8 (CH_2)	1.50 (m)	17	57.8 (CH)	1.22 (m)
4b		1.20 (m)	18	18.2 (CH_3)	0.92 (s)
5	38.2 (CH)	1.72 (m)	19	12.4 (CH_3)	0.69 (s)
6a	35.6 (CH_2)	1.51 (m)	20	40.4 (CH)	2.19 (dd, J = 15.5, 8.4)
6b		1.32 (m)	21	22.1 (CH_3)	1.08 (d, J = 6.6)
7	74.8 (CH)	4.00 (t, J = 2.5)	22	138.9 (CH)	5.26 (dd, J = 15.1, 8.8)
8	126.3 (C)		23	130.5 (CH)	5.11 (dd, J = 15.1, 8.8)
9	44.7 (CH)	1.97 (m)	24	52.1 (CH)	1.59 (m)
10	37.9 (C)		25	32.6 (CH)	1.57 (m)
11a	20.1 (CH_2)	1.64 (m)	26	21.3 (CH_3)	0.87 (dd, J = 6.2)
11b		1.52 (m)	27	19.3 (CH_3)	0.82 (dd, J = 6.2)
12a	37.2 (CH_2)	1.64 (m)	28a	26.1 (CH_2)	1.45 (m)
12b		1.16 (m)	28b		1.22 (m)
13	43.9 (C)		29	12.9 (CH_3)	0.84 (m)
14	149.3 (C)		OCH_3	54.3 (CH_3)	3.11 (s)

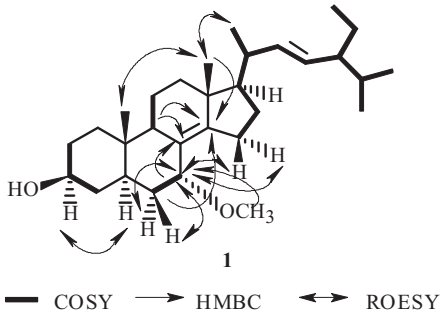
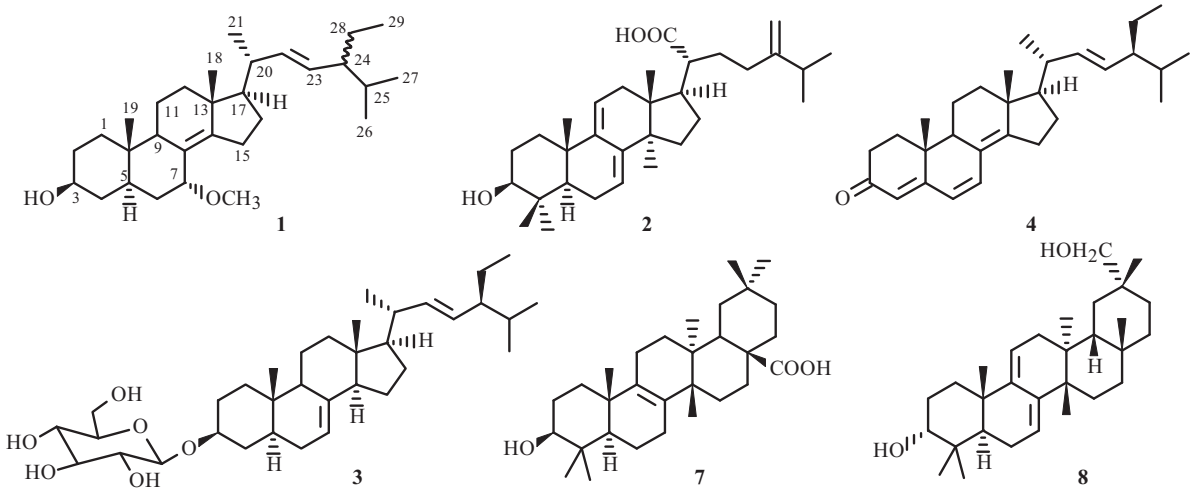


Fig. 1. COSY, HMBC, and ROESY correlations of **1**.

The known compounds were identified as dehydroeburicoic acid (**2**) [7], α-spinasterol-3-*O*-β-D-glucopyranoside (**3**) [4], stigmasta-4,6,8(14),22-tetraen-3-one (**4**) [8], ergosterol (**5**) [9], ursolic acid (**6**) [10], 3β-hydroxymultiflora-8-en-17-oic acid (**7**) [11], karounidiol (**8**) [12], 1*H*-indole-3-aldehyde (**9**) [13], methyl 1*H*-indole-3-carboxylate (**10**) [14], methyl 1*H*-indole-3-acetate (**11**) [13], *p*-hydroxybenzaldehyde (**12**) [15], isovanillin (**13**) [15], 2-hydroxybenzyl alcohol (**14**) [16], and 4-hydroxycinnamic acid (**15**) by comparison of their spectroscopic data with literature data or directly by spectroscopic data.



EXPERIMENTAL

Optical rotation was recorded on a Horiba SEPA-300 polarimeter. UV spectra were measured on a Shimadzu UV-2401PC spectrophotometer. IR spectra were obtained on a Tensor 27 spectrometer with KBr pellets. NMR spectra were recorded on a Bruker AV-400 or DRX-500 spectrometer. ESI-MS were recorded on a VG Auto Spec-3000 spectrometer, and HR-ESI-MS were determined on an API QSTAR Pulsar 1 spectrometer. Column chromatography (CC) was carried out on silica gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, China), RP-18 (40–60 μm; Daiso Co., Osaka, Japan), MCI gel CHP 20P (75–150 μm, Tokyo, Japan), and Sephadex LH-20 (Amersham Pharmacia, Uppsala, Sweden).

Plant Material. The roots of *C. sativus* were collected from Changsha County, Hunan Province, China, in July 2009, and authenticated by one of our authors (X. J. Zhou). A voucher specimen (ZHXJ-200912) was deposited at Hunan University of Chinese Medicine, Hunan Province, China.

Extraction and Isolation. The dried *Cucumis sativus* root powders (8 kg) were extracted with EtOH (2 × 40 L) to give an extract (570 g), which was suspended in water and partitioned by petroleum ether, EtOAc, and *n*-BuOH (each 3 × 6 L), respectively. The EtOAc-soluble extract (102 g) was fractionated using a silica gel column eluted with petroleum ether with increasing amounts of EtOAc to afford five fractions (Frs. 1-5). Fraction 2 (18.5 g) was divided into eight parts (Frs. 2-1–2-8) using an MCI gel CHP 20P column eluting with gradient aqueous MeOH. Fraction 2-7 (1.25 g) was chromatographed on a silica gel column ($CHCl_3$–Me_2CO, 100:3), followed by RP-18 (aqueous MeOH, 80–100%) and Sephadex LH-20 ($CHCl_3$–MeOH, 6:4) columns, to give compound **1** (38 mg). Fraction 2-8 (2.1 g) was submitted to vacuum liquid chromatography ($CHCl_3$–Me_2CO, 20:1), followed by gel filtration on Sephadex LH-20 (MeOH) to yield compounds **2** (8 mg) and **6** (6 mg). Fraction 2-6 (110 mg) was purified by RP-18 (aqueous MeOH, 50–100%) to give compounds **3** (31 mg), **5** (15 mg), **7** (36 mg), and **8** (20 mg). Fraction 2-4 (1.8 g) was fractionated by Sephadex LH-20 (MeOH) to afford two parts, one of which was purified by PTLC (petroleum ether–EtOAc, 12:5) to yield compound **11** (6 mg), and the other purified by a silica gel column (petroleum ether–EtOAc, 5:1) to yield compound **10** (10 mg). Fraction 2-3 (2.5 g) was passed through an MCI gel CHP 20P column eluting with gradient aqueous MeOH, followed by Sephadex LH-20 (MeOH) and final RP-18 columns (aqueous MeOH, 60–100%) to yield compounds **9** (10 mg) and **15** (12 mg). Fraction 2-2 (2.2 g) was passed through an RP-18 column (aqueous MeOH, 30–100%) and a silica gel column ($CHCl_3$–Me_2CO, 100:1), and finally purified by Sephadex LH-20 (MeOH) to give compounds **13** (9 mg) and **12** (10 mg). Fraction 2-1 (1.3 g) was submitted to Sephadex LH-20 (MeOH) and then PTLC ($CHCl_3$–Me_2CO, 5:1) to yield compound **14** (8 mg). Fraction 1 (3.9 g) was divided into three parts (Frs. 1-1–1-3) by Sephadex LH-20 ($CHCl_3$–MeOH, 6:4). Fraction 1-2 (1.35 g) was chromatographed using a silica gel column ($CHCl_3$–EtOAc, 100:0.2), a Sephadex LH-20 ($CHCl_3$–MeOH, 6:4) column, and finally an RP-18 column (aqueous MeOH, 80–100%) to give compound **4** (12 mg).

Stigmasta-8(14),22-diene-7α-methoxy-3β-ol (1). White amorphous solid; $[\alpha]_D^{21}$–23.2° (*c* 0.28, MeOH). UV (MeOH, λ_{max}, nm): 204 (4.12). IR (KBr, ν_{max}, cm^{-1}): 3433, 2956, 2928, 2870, 1633. For ^1H (400 MHz) and ^{13}C NMR (100 MHz) data, see Table 1. ESI-MS (negative) *m/z* 477 $[M + Cl]^-$; HR-ESI-MS (negative) *m/z* 477.3493 $[M + Cl]^-$ (calcd for $C_{30}H_{50}O_2Cl$, 477.3499).

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