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Echinopines A and B: Sesquiterpenoids Possessing an Unprecedented Skeleton from *Echinops spinosus*

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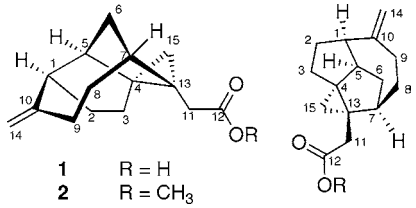
ABSTRACT



Echinopines A (1) and B (2), novel sesquiterpenoids with an unprecedented rearranged skeleton named echinopane, were isolated from the roots of *Echinops spinosus*. The structures were elucidated by extensive spectroscopic analysis. The relative configuration of 1 was assigned by a combination of NOESY correlations and a simulation analysis. A plausible biosynthetic pathway for echinopane was discussed.

The genus *Echinops* (Compositae) consists of ca. 100 species in the world. In China, this genus is represented by 17 species mainly distributed in the northwestern part.[1] A root of *E. spinosus* was brought from Morocco in 2003. Previous chemical investigation on the *Echinops* species demonstrated the presence of polyacetylene thiophenes,[2] an alkaloid,[3] a flavone glycoside,[4] and benzothiophene glycosides.[5] In continuation of our efforts to identify biologically active components from medicinal plants, extensive investigation of the constituents of the root of *E. supinosus* resulted in the isolation of two novel sesquiterpenoids with an unprecedented skeleton. We here describe their isolation and structure elucidation.

The air-dried roots of *E. spinosus* (3.0 kg) were cut into small pieces and then were exhaustively extracted with MeOH. The pooled methanolic extracts were concentrated under vacumm until most of the solvents were removed. The residue was then suspended in brine and partitioned successively with hexane, CH_2Cl_2, and EtOAc. An aliquot (30 g) of the CH_2Cl_2 extract was subjected to silica gel normal phase column chromatography (CC) to fractionation and eluted with hexane/acetone with increasing polarity from 10% to 80% acetone to yield 10 fractions, denoted as Fr.1−10. Fr.4 was

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(4) Ram, S. N.; Roy, R.; Singh, B.; Singh, R. P.; Pandey, V. B. *Planta Med.* **1996**, *62*, 187.
(5) Koike, K. K.; Jia, Z. H.; Nikaido, T.; Liu, Y.; Zhao, Y. Y.; Guo, D. A. *Org. Lett.* **1999**, *1*, 197−198.

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Table 1. The ^{1}H and ^{13}C NMR Data for **1** and **2** in $CDCl_3$ (500 MHz for ^{1}H, 125 MHz for ^{13}C)

	1					**2**		
position	δ_H (mult)[a]	J (Hz)	δ_C	HMBC	NOESY[b]	δ_H (mult)	J (Hz)	δ_C
1	2.81 (td)	9.1, 2.1	48.7	5, 4, 10, 14	$2a^w$, 5^s, $6b^w$, $14b^s$	2.80 (br t)	~10	48.5
2a	2.16 (om)		31.1	1, 3, 4, 5, 10	1^w, $2b^s$	2.14 (m)		30.9
2b	1.65 (dddd)	14.0, 9.7, 6.7, 2.6		1, 3, 4, 5, 10	$2a^s$, $9b^m$	1.65 (m)		
3a	1.95 (om)		25.8		$3b^s$, $11a^m$, $15b^w$	1.93 (m)		25.4
3b	1.52 (ddd)	13.5, 9.7, 4.0		1, 4, 5, 13	$3a^s$, $11b^s$	1.51 (m)		
4			41.5					29.8
5	2.26 (~t)	~8.0	48.6	1, 4, 7, 10, 13, 15	1^s, $6a^s$, $6b^s$, $15a^s$	2.25 (~t)	~9	48.4
6a	1.44 (dtd)	13.7, 7.4, 0.9	30.5	1, 5, 7, 8, 13	5^s, $6b^s$, 7^m, $15a^s$	1.44 (m)		30.3
6b	1.36 (d)	13.7		1, 4, 5, 7, 8, 13	1^w, 5^s, $6a^s$, 7^m	1.35 (d)	13.8	
7	2.43 (m)		40.6		$6a^m$, $6b^m$, $8a^m$, $8b^m$	2.39 (m)		40.4
8a	1.93 (om)		32.2		7^m, $8b^s$, $11a^m$	1.93 (m)		32.5
8b	1.27 (tdd)	13.6, 4.0, 3.0		7, 9, 10	7^m, $8a^s$, $9a^s$	1.26 (m)		
9a-eq	2.12 (odt)	13.3, ~4.7	29.4	1, 8, 10, 14	$8b^s$, $9b^s$, $14a^s$	2.11 (m)		29.3
9b-ax	1.82 (td)	13.3, 4.2		1, 7, 8, 10, 14	$2b^m$, $9a^s$, $11a^s$	1.818 (td)	13.2, 4.0	
10			154.3					154.3
11a	2.66 (dd)	15.3, 1.3	35.2	7, 12, 13, 15	$3a^m$, $8a^m$, $9b^s$, $11b^s$	2.65 (dd)	15.1, 1.3	35.1
11b	1.75 (d)	15.3		4, 7, 12, 13, 15	$3b^s$, $11a^s$, $15b^s$	1.72 (d)	15.1	
12			178.2					173.7
13			30.0					41.0
14a	4.63 (d)	2.6	112.1	1, 8, 9, 10	$9a^s$	4.63 (d)	2.6	111.9
14b	4.60 (d)	2.6			1^s	4.60 (d)	2.6	
15a	0.73 (dd)	5.3, 1.3	16.1	4, 5, 7, 11, 13	5^s, $6a^s$, $15b^s$	0.69 (dd)	5.1, 1.3	15.7
15b	0.50 (d)	5.3		3, 4, 5, 7, 11, 13	$3a^w$, $11b^s$, $15a^s$	0.47 (d)	5.1	
OH	10.53 (br)							
OMe						3.68 (s)		51.3

[a] Mutiplicity: s, singlet; d, doublet; t, triplet; m, mutiplet; o, overlapped. [b] NOESY intensities are marked as strong (s), medium (m), or weak (w).

applied to silica gel CC and RP-HPLC to afford compounds **1** (2.0 mg) and **2** (1.6 mg) (Figure 1).

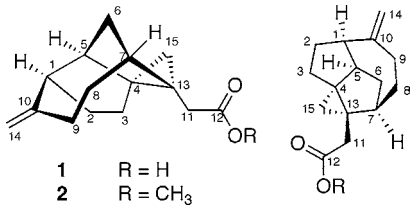


Figure 1. Structures of echinopines A and B.

The molecular formula of **1**,[6] $C_{15}H_{20}O_2$, was inferred from its HR-FAB-MS in which a sodiated molecular ion $[M + Na]^+$ was detected at *m*/*z* 255.1362 (calcd 255.1361). Six indices of hydrogen deficiency were calculated from the molecular formula. Analysis of the ^{13}C NMR data and 2D NMR spectral data (Table 1) disclosed 15 carbon signals. The ^{1}H NMR signals of **1** distributed mainly in the relative upfield region around δ 0.5−2.6 ppm. The ^{13}C NMR and HMQC spectra grouped all of the carbon signals into the following assemblies: one quaternary carbonyl carbon, three methines, seven methylenes, two quaternary sp^3 hybridized carbons, and two sp^2 hybridized carbons. The latter two (δ_C 112.1 and δ_C 154.3) were typical of an exocyclic double bond, which was supported by a pair of isolated AB signals, δ_H 4.63 (1H, d, J = 2.6 Hz, H-14a) and δ_H 4.60 (1H, d, J = 2.6 Hz, H-14b), in the ^{1}H NMR spectrum. These observations indicated that **1** contained 4 rings and the last proton was incorporated in a carboxy group (δ_H 10.53 and δ_C 178.2).[7] This is in good agreement with the fact that with further inspection of the ^{13}C NMR spectrum of **1**, no other carbons in **1** were found to be oxygenated except for the carbonyl. Interpretation of $^{1}H-^{1}H$ COSY and HMBC spectra (Figure 2) permitted the construction of connected protons as well as the positional assignment of functional groups and quaternary carbons. In the $^{1}H-^{1}H$ COSY spectrum, the connectivity from H-1 to H-2-H-3 and from H-1 to H-5-H-6-H-7-H-8-H-9 was deduced. Long-range correlations from H-5 and H-8 to C-10, H-1 and H-9 to C-14 as well as H-14 to C-1 and C-9 in the HMBC spectrum (key correlations depicted in Figure 2) suggested the exo-cyclic double bond was located at C-10 and C-14. This compound also displayed a discrete spin system at high field of δ 0.73 (1H, dd, J = 5.3, 1.3 Hz, H-15a) and δ 0.50 (1H, d, J = 5.3 Hz, H-15b)

(6) Echinopine A (**1**): colorless solid; $[\alpha]^{22}_D$ +23 (*c* 0.11, $CHCl_3$). ^{1}H NMR and ^{13}C NMR ($CDCl_3$): see Table 1.

(7) Huang, X. H.; Soest, R.; Roberge, M.; Andersen, R. *Org. Lett.* **2004**, *6*, 75−78.

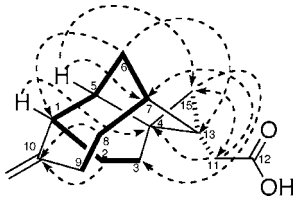


Figure 2. Dashed arrows denote the key HMBC correlations (H→C) of **1**. Solid bold lines indicate the connectivities (dashed bonds mean long-range correlation) deduced by the ^{1}H−^{1}H COSY correlations.

associated with the cyclopropyl methylene group.[8] These protons have a scalar geminal coupling constant of $J = 5.3$ Hz that could be compatible with a 3-membered ring.

In addition, since these are not coupled with other protons, the cyclopropane should be highly substituted. Typical AB spin systems at δ 2.66 (1H, dd, $J = 15.3$, 1.3 Hz, H-11a) and δ 1.75 (1H, d, $J = 15.3$ Hz, H-11b) with a large geminal coupling constant implied a methylene moiety. The signal at δ 2.66 also displayed a long-range coupling with the signal at δ 0.73 in the ^{1}H−^{1}H COSY map, which inferred that this methylene appendage was attached to the cyclopropane ring. The methylene protons showed long-range correlations with C-4, C-13, and C-15 as well as the carbonyl carbon at δ 178.2 revealing that the carboxy group was connected with C-11. The long-range correlations between H-15 and C-3, C-4, C-5, C-7, C-11, C-13; and H-5 to C-1, C-4, C-7, C-10, C-13, C-15; as well as H-3 to C-1, C-4, C-5, C-13; and H-11 to C-4, C-7, C-12, C-13, C-15 demonstrated a tetrasubstituted cyclopropane moiety at C-4 and C-13. This conclusion was in full agreement with the readout of the HMBC spectrum in which correlations were observed between H-6 and C-1, C-4, C-5, C-7 C-8, C-13. Only H-11a and H-11b exhibited HMBC correlations with the carboxy carbon clearly indicating that a carboxymethyl group connected to a quaternary carbon was positioned at C-13. Taking all above arguments together, the structure of **1** was, therefore, characterized as shown in Figure 1, to which we gave a trivial name echinopine A according to its originality, representing the first member of a new family of tetracyclic sesquiterpene with a rearranged carbon framework (echinopane skeleton). The skeleton name of **1** is echinop-10(14)-en-12-oic acid.[9]

The relative configuration of **1** was defined on the basis of the phase sensitive NOESY spectrum as shown in a three-dimensional drawing (Figure 3), which was generated by MM2 calculation.[10] The 7-membered ring seems to adopt a flat chair form. Two 5-membered rings (C-4-C-5-C-6-C-7-C-13) and (C-1-C-2-C-3-C-4-C-5) are an envelope shape.

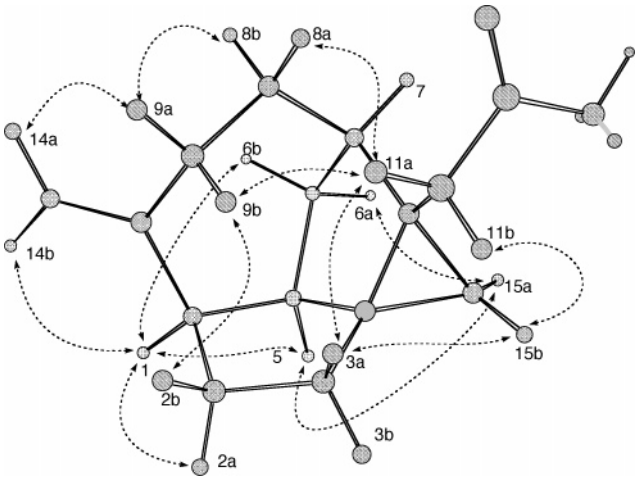


Figure 3. Calculated conformation by MM2 and significant NOESY correlations of compound **1**.

The failure to crystallize, limitation of material, and scarcity of sample source make compound **1** inaccessible to X-ray crystallographic analysis and chemical conversion to further characterize the structure. Nevertheless, these difficulties can be overcome through the unambiguous rationalization of all of the ^{1}H and ^{13}C NMR resonances by using a combination of ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY techniques.

To the best of our knowledge, compound **1** represents the first example of a novel 3/5/5/7-membered ring carbon framework. The proposed tetracyclic structure embodying a tetrasubstituted cyclopropane moiety is entirely consistent with its spectral properties and was chemically and biogenetically reasonable. A plausible biogenetic pathway from a guaiane skeleton to echinopine A was proposed (Scheme 1). This sesquiterpenoid appears to be synthesized from a guaiane type precursor **3**. Hydration of the 11(13)-double bond followed by C7(11→13) rearrangement gives enoic acid **4**.[11] Successive C15−C13 and C13−C4 cyclization steps construct the skeleton **1**. This compound is of great interest for its biogenetic pathway.

The molecular formula of compound **2**[12] was determined to be $C_{16}H_{22}O_2$ on the basis of the accurate sodiated-molecular ion peak at *m*/*z* 269.1518 (calcd 269.1517) in the HR-FAB-MS analysis, indicating six degrees of double-bond equivelance and 14 mass units heavier than that of **1**. Similar NMR studies were also conducted for **2**. The ^{1}H NMR spectroscopic pattern of **2** showed the same characteristics as that of compound **1**. Further comparison of the ^{13}C NMR data obtained for **2** (Table 1) with the data obtained for **1** showed that the molecules of **1** and **2** were closely related. The major difference in the ^{1}H NMR of **2** compared to **1** was the presence of an additional methyl group in **2**, which

(8) (a) Kouno, I.; Hirai, A.; Fukushige, A.; Jiang, Z. H.; Tanaka, T. *J. Nat. Prod.* **2001**, *64*, 286−288. (b) Brito, I.; Gueto, M.; Dorta, E.; Darias, J. *Tetrahedron Lett.* **2002**, *43*, 2551−2553.

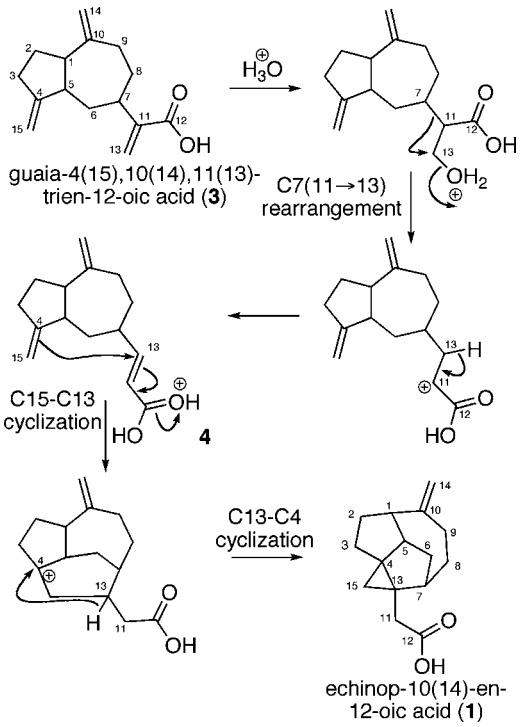
(9) The IUPAC name for **1**: (1*R**,2*R**,4*R**,7*R**,12*R**)-8-methylenetetracyclo[5.3.2.0^{2,4}.0^{4,12}]dodec-2-ylacetic acid.

(10) Molecular modeling calculations were performed by using the MM2 force field implemented in the Chem3D program V5.0 (Cambridge-Soft, Cambridge, MA). A conformational search was carried out by minimizing energy using standard MM2 constants based on the structure elucidated by the NOESY data.

(11) Su, B.-N.; Takaishi, Y.; Yabuuchi, T.; Kusumi, T.; Tori, M.; Takaoka, S. *Tetrahedron Lett.* **2000**, *41*, 2395−2400.

(12) Echinopine B (**2**): colorless solid; $[\alpha]^{22}_D$ +21 (*c* 0.14, $CHCl_3$). ^{1}H NMR and ^{13}C NMR ($CDCl_3$): see Table 1.

Scheme 1. Plausible Biosynthetic Pathway for the Echinopane Skeleton



was suggested by the spectral data at δ_H 3.68 (3H, s). Its chemical shift inferred that it should be bonded to an oxygen atom as a typical oxymethyl residue. This postulate was in agreement with the proposed molecular formula, which was further corroborated by the ^{13}C NMR spectrum as an additional signal compared to **1** was detected at δ_C 51.3.[13] In addition, the ^{13}C NMR spectrum of **2** indicated that the carbonyl resonance was moved from δ_C 178.2 in **1** upfield to δ_C 173.7 in **2**, which demonstrated that the carboxy group in **1** was esterified in **2**. This conclusion was further evidenced by the absence of the broad signal of carboxylic acid proton in the ^1H NMR spectrum of **2**. Taking all these spectral features into account, the structure of **2** was elucidated unequivocally as shown in Figure 1, accordingly giving a trivial name echinopine B. The full ^1H and ^{13}C NMR assignments were established by means of a combination of COSY, HMQC, HMBC, and NOESY spectral measurements.

Both **1** and **2** were inactive in vitro cytotoxicity screen against the human breast cancer MCF-7 cell line. Further investigation of the minor constituents of this plant and biological evaluation of the sesquiterpenoids by using different assay systems are in progress.

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Supporting Information Available: ^1H, ^{13}C, COSY, HSQC, HMBC, and NOESY NMR spectra of echinopine A (**1**), HMBC and NOESY data of echinopine B (**2**), and their isolation procedure. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) (a) Marrero, J.; Rodriguez, A. D.; Baran, P.; Raptis, R. G. *Org. Lett.* **2003**, *5*, 2551−2554. (b) Blas, B.; Zapp, J.; Becker, H. *Phytochemistry* **2004**, *65*, 127−137. (c) Morita, H.; Ishioka, N.; Takatsu, H.; Shinzato, T.; Obara, Y.; Nakahata, N.; Kobayashi, J. *Org. Lett.* **2005**, *7*, 459−462.