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CYTOTOXIC DITERPENOIDS FROM *ISODON MEGATHYRSUS*

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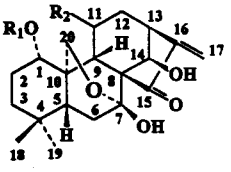
ABSTRACT.—A new diterpenoid, megathyrin A, together with three known compounds, rabdocoetsins B, C, and D, were isolated from the leaves of *Isodon megathyrsus*, and their structures and nmr spectral data were assigned by a combination of one- and two-dimensional nmr techniques. These compounds displayed significant cytotoxic activity.

The genus *Isodon* (family Labiatae) comprises 150 species, of which more than 100 are distributed in China. About 10 species of this genus have been used in Chinese traditional medicine for gastrointestinal disorders and as antitumor and antiphlogistic agents (1,2). Our previous studies on the plants of this genus led to the isolation of more than one hundred new diterpenoids (3,4). As a continuation of these studies, we have investigated *Isodon megathyrsus* (Diels) H.W. Li, a plant growing in the northwestern area of Yunnan Province, People's Republic of China, which has not been previously investigated chemically. This paper deals with the isolation and structure identification of a new diterpenoid, megathyrin A [**1**], the unambiguous nmr spectral assignments of the known diterpenoids, rabdocoestins B [**2**], C [**3**], and D [**4**] (5,6), through a series of one- and two-dimensional nmr techniques, including COSY [DQF-COSY (7,12)], phase-sensitive ROESY (8–10), FLOCK (11), HMQC (12) (or HETCOR) and HMBC (10,12) [or FLOCK (11)] nmr techniques, and the demonstration of the potent cytotoxic activity of these compounds.

RESULTS AND DISCUSSION

The MeOH extract of the leaves of *I. megathyrsus* was subjected to repeated chromatography to yield megathyrin A [**1**] and rabdocoetsins B [**2**], C [**3**], and D [**4**].

Megathyrin A [**1**], colorless crystals, $C_{20}H_{28}O_5$ (hrfabms), showed a conjugated ketone (1726, 1647 cm^{-1}) in its ir spectrum, which was also supported by the uv absorption (230.5 nm). The ^1H-, ^{13}C-, DEPT, and APT nmr spectra of **1** showed the presence of two tertiary methyls, seven methylenes (including an oxygenated methylene and an exo-methylene group), five methines (including two oxygenated methines), four quaternary carbon atoms, a hemiacetal carbon atom, a ketonic carbon, and three hydroxy functions, which led to the conclusion that megathyrin A [**1**] possessed an *ent*-7α,20-



1 $R_1=R_2=H$
2 $R_1=Ac$, $R_2=H$
3 $R_1=H$, $R_2=OH$
4 $R_1=H$, $R_2=OAc$

epoxy-kaurene-hemiacetal-15-one skeleton. This notion was further confirmed by its DQF-COSY, HMQC, HMBC, and FLOCK nmr spectra. Inspection of the double-quantum filtered COSY (DQF-COSY), and HMQC spectra of **1** established the existence of the following three fragments: -CH(OH)CH_2CH_2-, -$CHCH_2CO$(OH)-, and -$CHCH_2CH_2CHCH$(OH)-, bearing quaternary carbon or oxygen atoms at both ends, and suggested that the hydroxy functions should be located at C-1, C-14, and C-7. This intimation was unambiguously confirmed by the proton-carbon long-range chemical shift correlation 2D nmr technique (FLOCK), and reverse-detected heteronuclear correlation 2D nmr technique (HMBC), both of which served to assign the quaternary carbons and establish the skeleton; the major results are shown in Table 1. In these two experiments, $J=6$ Hz was used for the proton-carbon long-range coupling constant, and most of the protons were shown to have correlations with the same carbons in both experiments. However, some protons showed quite different results in these experiments. For example, the protons 9β, 1β, and 5β showed clearer correlations with more carbons in the HMBC than in the FLOCK experiment, whereas the 6β and 13α protons showed correlations with more carbons in the FLOCK than in the HMBC experiment. By inspection of the results (Table 1) from both experiments, the skeleton and all of the nmr data could be assigned completely and unambiguously as shown in the structure of **1** and in Tables 2 and 3. The determination of the positions for the epoxy hemiacetal, the double-bond, and the carbonyl groups will be briefly described here.

The FLOCK and HMBC spectra of **1** showed that the H-20a signal at δ 4.49 was coupled to C-5 (δ 48.63) and C-10 (δ 41.07), and the H-20b signal at δ 4.75 was coupled

TABLE 1. Principal Results from the ROESY, HMBC, and FLOCK Nmr Spectra of Megathyrin A [**1**].[a,b]

Proton	ROESY (Proton)	FLOCK (Carbon)	HMBC (Carbon)
1β	2β, 3β, 5β, 9β	(10), 20	5, 9, (10), 20
2α	19, 20b	n.o.	(1)
2β	1β, 3α, 3β	n.o.	(1)
3α	2β, 3β, 18	1, (2)	(2)
3β	1β, 2β, 3α, 18	n.o.	(2)
5β	1β, 6α, 6β, 9β, 18	(6), (10), 7, 20	(4), (6), 9, (10), 18, 19
6α	5β, 6β, 18, 19	(7), 10	(5), (7), 10
6β	5β, 6α	(7), 10	n.o.
9β	1β, 9β	n.o.	5, (10), (11), 20
11α	11β, 14α, 20a	(12)	(9)
11β	11α, 12β	(12)	8, 13
12α	12β, 13α	16	9, (13)
12β	11α, 12α, 13α	(11), 16	(11), (13), 14
13α	12α, 14α	8, (11), 14, 16, 17	15
14α	11α, 20a	15, 16	7, 15, 16
17a	17b	13, 15, (16)	13, 15, (16)
17b	17a	13, 15	13, 15, (16)
18	3α, 3β, 5β, 6α	3, (4), 5, 19	3, (4), 5, 19
19	2α, 20b	3, (4), 5, 18	3, (4), 5, 18
20a	11α, 14α	5, (10)	5, (10)
20b	2α, 19	7, 9	7, 9

[a]The ROESY experiment was performed at 500.1 MHz with a spin-lock time of 300 msec, and a spin-lock field strength of 5 kHz (13,14).

[b]The FLOCK and HMBC experiments were performed at 500.1/125.8 MHz with $J=6$ Hz (13,14); two-bond correlations are in parentheses; n.o. indicates no clear FLOCK or HMBC contours were observed for this proton.

TABLE 2. ^{1}H-Nmr Data of the Diterpenoids **1–4** from *Isodon megathyrsus*.[a]

Proton	Compound			
	1	**2**	**3**	**4**
1β	3.59 (dd, 12.5, 3.0)	4.72 (dd, 11.5, 5.0)	4.05 (dd, 11.0, 5.5)	3.96 (dd, 10.5, 5.5)
2α	1.79 (m)	1.73 (m)	1.89 (m)	1.87 (m)
2β	1.78 (m)	1.43 (m)	1.80 (m)	1.80 (m)
3α	1.36 (m)	1.26 (m)	1.38 (dt, 13.5, 4.5)	1.37 (m)
3β	1.18 (m)	1.18 (m)	1.20 (dd, 13.5, 13.0)	1.20 (ddd, 13.0, 13.0, 4.0)
5β	1.67 (dd, 11.0, 7.5)	1.47 (m)	1.56 (dd, 11.5, 6.5)	1.56 (dd, 10.5, 5.5)
6α	1.98 (dd, 12.5, 7.5)	1.95 (dd, 13.5, 7.5)	1.98 (dd, 13.5, 6.5)	1.96 (dd, 12.5, 5.5)
6β	3.55 (dd, 12.5, 11.0)	3.56 (dd, 13.5, 13.3)	3.63 (dd, 13.5, 12.0)	3.59 (12.5, 10.5)
9β	1.80 (m)	1.77 (m)	1.82 (d, 10.0)	2.06 (d, 9.5)
11α	2.35 (m)	1.99 (m)	4.75 (m)	5.93 (m)
11β	1.87 (m)	1.14 (m)	—	—
12α	2.39 (dt, 16.5, 8.5)	2.30 (dt, 14.0, 9.0)	2.78 (ddd, 13.5, 10.0, 8.3)	3.21 (dt, 14.5, 9.5)
12β	1.54 (dd, 16.5, 9.5)	1.49 (m)	1.64 (dd, 13.5, 10.0)	1.29 (dd, 14.0, 9.0)
13α	3.10 (d, 8.5)	3.06 (d, 9.5)	2.97 (dd, 10.0, 3.0)	2.91 (dd, 10.0, 4.0)
14α	5.30 (br s)	5.22 (br s)	2.42 (d, 12.5)	2.24 (d, 12.5)
14β	—	—	2.36 (dd, 12.5, 3.0)	2.36 (12.5, 4.0)
17a	6.22 (br s)	6.21 (br s)	5.92 (br s)	5.92 (br s)
17b	5.39 (br s)	5.35 (br s)	5.19 (br s)	5.18 (br s)
18	0.76 (s)	0.70 (s)	0.71 (s)	0.73 (s)
19	1.06 (s)	1.01 (s)	1.06 (s)	1.06 (s)
20a	4.49 (br d, 10.5)	4.41 (br d, 10.0)	4.55 (d, 9.5)	4.49 (dd, 11.0, 2.0)
20b	4.75 (br d, 10.5)	4.50 (br d, 10.0)	4.72 (d, 9.5)	4.67 (d, 11.0)
OAc	—	1.96 (s)	—	1.99 (s)

[a]Recorded in pyridine-d_5, chemical shift values are reported as δ values (ppm) from TMS at 500.1 MHz; signal multiplicity and coupling constants (Hz) are shown in parentheses.

to C-9 (δ 54.06) and C-7 (δ 98.25). The latter correlation indicated that H-20 and C-7 should be three bonds away, i.e., that C-20 and C-7 should be connected to the same atom, which led to the assignment of the epoxy hemiacetal linkage between C-20 and

TABLE 3. ^{13}C-Nmr Data of the Diterpenoids **1–4** from *Isodon megathyrsus*.[a]

Carbon	Compound			
	1	**2**	**3**	**4**
1	73.30	76.05	73.72	72.57
2	30.37	25.40	28.38	28.39
3	38.83	38.03	39.48	39.01
4	33.92	33.74	34.27	34.16
5	48.63	48.69	48.74	48.75
6	32.77	32.55	32.59	32.46
7	98.25	98.12	96.02	96.05
8	59.82	59.29	57.48	57.08
9	54.06	52.65	57.48	54.21
10	41.07	39.53	42.14	41.68
11	20.35	18.48	63.37	67.65
12	31.36	30.95	39.48	36.65
13	43.29	43.14	34.27	34.16
14	73.23	73.22	26.77	27.82
15	203.95	203.86	205.56	205.57
16	153.90	153.55	154.69	154.72
17	116.57	116.93	113.69	113.68
18	31.69	31.39	31.88	31.82
19	20.61	20.35	21.10	20.63
20	64.18	63.75	64.98	65.01
$COCH_3$	—	170.03	—	170.26
$COCH_3$	—	21.37	—	21.11

[a]Recorded in pyridine-d_5; chemical shifts are reported as δ (ppm) from TMS at 125.8 MHz.

C-7. The alternative that they could be five bonds away, through C-20, C-10, C-5 and C-6, or C-20, C-10, C-9, and C-8, was regarded as out of the range to produce FLOCK and HMBC correlations. Carbon-15 should be the carbonyl carbon from its three-bond correlations with H-17 and H-14 in the FLOCK and HMBC nmr spectra of **1**, and the double bond should be at C-16, C-17 and connected to C-13 based on the correlations between H-12 and C-16, and H-13 and C-17 in its FLOCK spectrum, and H-17 and C-13, and H-14 and C-16 in both the FLOCK and HMBC spectra of **1**. Thus, all of the ^{13}C-nmr data were unambiguously assigned as shown in Table 3.

The unambiguous assignment of the stereochemistry of **1** was achieved by a combination of a phase-sensitive 2D nOe (ROESY) nmr experiment and computer modeling calculations. The major results are shown in Table 1 and in Figure 1. Based on the information from the ^{1}H-, COSY, and ROESY nmr results, a computer-assisted three-dimensional structure was obtained by the molecular modeling program PCMODEL 386 V 4.0, using MMX force field calculations for energy minimization. This structure shows the six-membered rings A, B, and C in a chair, boat and boat conformation, respectively. The distances between H-20a and H-14α (2.49 Å), H-20a and H-11α (2.23 Å), and H-20b and H-2α (2.44 Å) were less than 2.50 Å, which is in the range to produce a nOe effect. In fact, prominent ROESY correlation contours between each of the above-described pairs were observed, and were used to support the presentation of the rings A, B, and C of **1** in a chair, boat and boat conformations, respectively, as shown in Figure 1. This structure shows two hydroxy groups in C-1α and C-14β orientations based on the observation of the nOe correlation contours between H-1β and H-3β (2.44 Å), H-1β and H-5β (2.74 Å), H-1β and H-9β (2.54 Å), as well as H-11α and H-14α (2.22 Å). The modeling calculations confirmed that all of the distances between each of the remaining proton pairs showing ROESY contours (Table 1) were less than 2.60 Å. This structure also generated information concerning the average dihedral angle and the corresponding coupling constants (J value) between vicinal protons, which were consistent with the observed J values from the nmr measurements. These data led to the confirmation of the stereochemical assignments of the protons and the functional groups, and thus to the complete assignment of the stereochemical features of this compound.

As an example, it was difficult to assign the H-6 protons based on the results from the ROESY spectrum, in which both of the H-6 protons showed a clear nOe effect with H-5β, but they were assigned by modeling calculations of the J values. Thus, H-6β appeared in front of the plane of the structure, and had a dihedral angle of 18° and a calculated J value of about 9.45 Hz with H-5β, and should have its signal at δ 3.55 as a double doublet with J=12.5 and 11.0 Hz, but its partner proton H-6α, having a dihedral angle of 134°, and J value of 6.9 Hz with H-5β, should be assigned to δ 1.98, a double doublet (J=12.5 and 7.5 Hz) in the ^{1}H-nmr spectrum. Furthermore, other observed J values from the ^{1}H-nmr measurements were consistent with the data from the modeling calculations. Thus, megathyrin A [**1**] was identified as 1α,7β,14β-

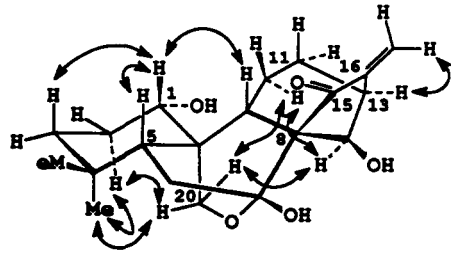


FIGURE 1. Expression of the major ROESY correlations in megathyrin A [**1**].

trihydroxy-*ent*-7β,20-epoxy-kaur-16-en-15-one; the unambiguous stereochemical assignments of the ^{1}H-nmr data are as shown in Table 2.

Rabdocoetsin B was previously reported to have the structure **2**, which is the 1-*O*-acetyl derivative of **1**, and was previously isolated from *I. coetsa* in 1990 (5), and in 1993, when collected in a different location (6). Its ^{1}H- and ^{13}C-nmr data were assigned by comparison with those of **1**, and the analyses of its ^{1}H-, ^{13}C-, DEPT, COSY, and HETCOR nmr spectra are shown in Tables 2 and 3. Comparison with Ref. (6) shows that the ^{13}C-nmr data for C-6, C-12, C-19, and the methyl carbon of the acetyl, as well as some of the ^{1}H-nmr data, need to be revised as shown.

Compounds **3** and **4** were identified as rabdocoetsins C and D, respectively, which were first reported in 1990 (5). However, rabdocoetsin C was named rabdocoetsin A on its second isolation by Y.-L. Xu *et al.* (6). In this report, it should be indicated that rabdocoetsin A and C are the same compound, and we have now used the first assigned name (rabdocoetsin C) for this compound. The complete and unambiguous nmr assignments of **3** and **4** were achieved by a combination of one- and two-dimensional nmr techniques (DEPT, APT, COSY, ROESY, HETCOR, and FLOCK), and are shown in Tables 2 and 3. Comparison with data of Xu and Kubo (6) indicated that the ^{13}C-nmr data for C-2, C-6, and C-14, as well as several ^{1}H-nmr data, should be revised as reported here.

Compounds **1**, **2**, **3**, and **4** were subjected to anticancer, antimalarial, and HIV reverse transcriptase inhibitory tests (15–18). All of the isolates showed potent cytotoxic activities as shown in Table 4, but none showed any activity in the antimalarial and HIV RT inhibitory tests.

TABLE 4. Evaluation of the Cytotoxic Activity of Compounds **1–4**.[a]

Compound	Cell Line (ED_{50}, μg/ml)[b]						
	Lu-1	KB	KB-V (+VLB)	KB-V (−VLB)	LNCaP	ZR-75-1	ASK[c]
Megathyrin A [**1**]	3.4	1.1	8.9	19.1	1.8	1.8	−
Rabdocoestin B [**2**]	0.5	0.3	7.2	>20	0.6	0.8	−
Rabdocoestin C [**3**]	0.4	0.6	>20	>20	0.7	1.0	−
Rabdocoestin D [**4**]	0.9	0.5	12.1	9.5	0.8	0.8	−
Colchicine[d]	0.2	0.2	0.6	3.5	0.06	0.1	+
Ellipticine[d]	0.2	0.04	0.2	0.3	0.8	0.9	−

[a]Key to cell lines used: Lu-1, human lung cancer; KB, human oral epidermoid carcinoma; KB-V, vinblastine-resistant KB (±VLB, vinblastine 1 μg/ml); LNCaP, hormone-dependent human prostate cancer; ZR-75-1, hormone-dependent human breast cancer; ASK, human astrocytoma.

[b]Positive limits: ED_{50} <5.0 μg/ml for Lu-1, KB, KB-V (+VLB), KB-V (−VLB), LNCaP, and ZR-75-1.

[c]"+" or "−" indicates presence or absence of the activity in the ASK test at the concentration of 100 μg/ml.

[d]Control compound.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Kofler hot-stage apparatus and are uncorrected. Uv spectra were measured on Beckman DU-7 and Phillips Pye Unicam PU8800 spectrophotometers, and the ir spectra were taken on Perkin-Elmer 577 and 983G instruments and recorded in KBr pellets. Optical rotations were taken with a Perkin-Elmer 241 and a Jasco 20C polarimeter, and ms were determined on DX-300 and Finnigan MAT-90 instruments in the ei-mode. ^{1}H-, ^{13}C-, DEPT, COSY, DQF-COSY, ROESY, HETCOR, HMQC, FLOCK, and HMBC nmr spectra were taken on a GE Omega 500 instrument operating at 500.1 MHz for ^{1}H- and homonuclear 2D nmr spectra, 125.8 MHz for ^{13}C- and DEPT nmr spectra, and 500.1/125.8 MHz for heteronuclear 2D spectra with a long-range coupling constant of J=6 Hz, using standard GE programs in pyridine-d_5 solution, and have been described in detail previously (13,14).

PLANT MATERIAL.—The leaves of *Isodon megathyrsus* were collected from Fugong County, Yunnan Province, People's Republic of China, in 1989, and identified by Prof. H.-W. Li. A voucher specimen was deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Academia Sinica, Kunming, People's Republic of China.

EXTRACTION AND ISOLATION.—The powdered, air-dried leaves (230 g) of *I. megathyrsus* were extracted with MeOH (0.5 liter×3) under reflux. The combined MeOH extract was concentrated *in vacuo*, and the residue (31 g) was directly chromatographed over Si gel (700 g), using petroleum ether with increasing proportions of $CHCl_3$, $CHCl_3$-MeOH (9.5:0.5, 9:1, 8:2, and 6:4), and Me_2CO, gave megathyrsin (**1**, 510 mg, 0.22%), rabdocoetsin B (**2**, 980 mg, 0.43%), rabdocoetsin C (**3**, 1300 mg, 0.57%), and rabdocoetsin D (**4**, 830 mg, 0.36%). Compounds **2–4** were identified by direct comparison with authentic samples through tlc, mixed mp, ir, and ^{1}H-nmr determinations.

Megathyrin A [**1**].—Compound **1** was obtained as colorless crystals (510 mg, 0.22%); mp 180–182°; $[\alpha]_D$ −22.5° (c=0.082, MeOH); uv (MeOH) λ max (log ε) 230.5 (4.05) nm; ir (KBr) ν max 3404, 1726, 1647, 1469, 1454, 1366, 1159, and 1060 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Tables 2 and 3; eims *m/z* 348 (M^+, 9), 331 (22), 330 (53), 315 (13), 312 (11), 302 (13), 275 (18), 249 (41), 203 (58), 208 (100), and 105 (98); hrms, observed 348.1937 for $C_{20}H_{28}O_5$, calcd 348.1931.

CYTOTOXICITY, ANTIMALARIAL, AND HIV-1 RT INHIBITORY ASSAYS.—The biological evaluations for cytotoxic, antimalarial, and HIV-1 RT inhibitory activities of these compounds were carried out according to established protocols (15–18).

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