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SPIRO-LACTONES, HYPEROLACTONE A–D FROM *HYPERICUM CHINENSE*

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Key Word Index—*Hypericum chinense*; Guttiferae; spiro-lactone; hyperolactone.

Abstract—Novel spiro compounds, hyperolactones A–D were isolated from stems and leaves of *Hypericum chinense*. Hyperolactones have a common spiro-lactone structure with a 2-alkyl- or 2-aryl-9-methyl-9-vinyl-1,7-dioxaspiro [4.4] non-2-ene-4,6-dione skeleton.

INTRODUCTION

In Asian countries, plants of the Guttiferae family have been used for folk medicines, dyes, antiseptics, fruits, and timbers for a long time. Many biologically active compounds have been isolated from the plants of the Guttiferae family and most of them have an acylphloroglucinol moiety [1–6]. Previously we reported the isolation of a unique spiro-compound, hyperolactone [7] together with the antiviral acylphloroglucinols [8, 9], chinesin I and II from *Hypericum chinense* L. [10]. The structure of hyperolactone was deduced by spectroscopic experiments, chemical transformations and finally by X-ray crystallography. In order to speculate the biosynthetic route of the unique compound, hyperolactone, we have studied the minor constituents of *H. chinense*. In the present work we describe the isolation and structure elucidation of hyperolactones A–D from *H. chinense*. The biosynthetic route of hyperolactones A–D is speculated upon and the numbering of hyperolactone presented in a preliminary report [7] is now revised because a common skeleton was found for hyperolactones A–D.

RESULTS AND DISCUSSION

The methanol extract of the leaves and stems was concentrated and the residue partitioned between ethyl acetate and water. The organic layer was evaporated and the residue subjected to silica gel column chromatography. The crude fractions were purified by HPLC to give hyperolactone [7], which was renamed hyperolactone A (**1**), and its related novel three compounds which were named hyperolactone B (**2**) (2 mg), C (**3**) and D (**4**), respectively.

Compound **2** had the molecular formula of $C_{13}H_{16}O_4$. The IR (1795, 1690, 1640 cm^{-1}), UV [203 nm (ε 1.4×10^4), 267 nm (ε 2.0×10^4)], ^1H and ^{13}C NMR spectra of **2** were very similar to those of **1**. The differences of the molecular formula and the ^1H and ^{13}C NMR spectra between the two compounds indicated that **2** had an isopropyl group instead of a 1-methylpropyl group as found in **1**. The stereochemistry of **2** was deduced from the comparison of the ^1H NMR chemical shifts and the NOE in **1** and **2**. In hyperolactone B (**2**), a NOE was observed between the 3-methyl group (δ1.24) and a lower field hydrogen (Ha-9, δ4.71) of the 9-methylene, whereas in **1**, a NOE was observed between the 3-methyl group (δ1.41) and a higher field hydrogen (Ha-9, δ4.05). These results indicated that a carbonyl group and an oxygen at C-4 of **2** were bonded in the opposite configuration as compared with **1**. The Ha-9 of **2** should be deshielded more strongly than the Ha-9 of **1** by the 5-carbonyl group.

Compound **3** had the molecular formula of $C_{16}H_{14}O_4$. The IR (1780, 1710, 1645, 1600 cm^{-1}), ^1H and ^{13}C NMR spectra of **3** suggested that **3** had a spiro-structure with similar functional groups as in **1** and **2**. The molecular formula, UV [254 nm (ε 1.4×10^4), 307 nm (ε 2.6×10^4)], and the ^1H and ^{13}C NMR spectra indicated that **3** had a phenyl group instead of a 1-methylpropyl group as in **1**. The HMBC of **3** allowed the assignment of all the carbons and confirmed the structure. The stereochemistry of **3** was deduced from similar NOE experiments as conducted in the case of **1** and **2**. For hyperolactone C, a NOE was observed between a higher field hydrogen (δ4.12) of the 9-methylene and 3-methyl group (δ1.53), which was similar to the result obtained with hyperolactone A (**1**).

Compound **4** had a molecular formula of $C_{16}H_{16}O_4$. The ^1H and ^{13}C NMR showed similar spectra to **3**, except for a pair of methine signals (^1H NMR δ 3.29 and 3.47; ^{13}C NMR δ58.6 and 61.0). The IR spectrum (3400 cm^{-1}, br) showed a hydroxy group. These facts indicated that **4** had a very similar structure to hyperolactone C (**3**), but the enol ether ring was reduced and opened to give C-4 diastereomers of the enol.

The common carbon skeleton structure in the hyperolactones may be biosynthesized from isopentenyl pyro-

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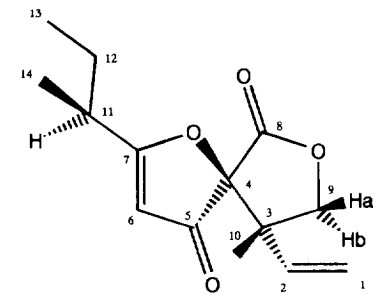

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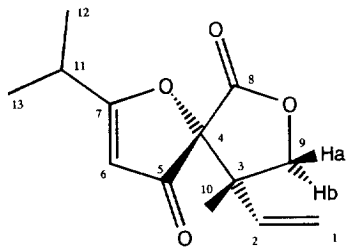

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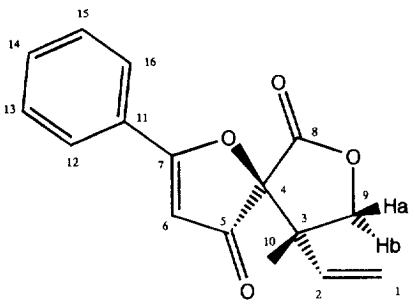

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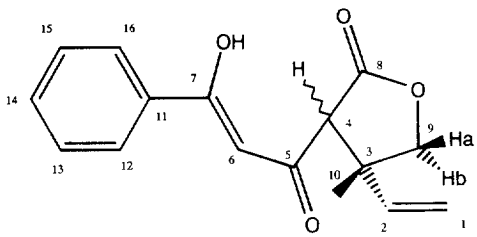

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Table 1. ^{1}H NMR spectral data of four hyperolactones

C	Hyperolactone A	Hyperolactone B	Hyperolactone C	Hyperolactone D
1	5.25 *d* (17)	5.27 *d* (18.0)	5.26 *d* (17.8)	5.22 *d* (17.6), 5.24 *d* (17.6)
	5.28 *d* (11)	5.30 *d* (11.5)	5.28 *d* (10.5)	5.23 *d* (11.5), 5.28 *d* (11.5)
2	5.93 *dd* (17, 11)	5.93 *dd* (18.0, 11.5)	6.00 *dd* (17.8, 10.5)	5.91 *dd* (17.6, 11.5)
				6.01 *dd* (17.6, 11.5)
4				3.29 *s*, 3.47 *s*
6	5.38 *s*	5.44 *s*	5.99 *s*	6.29 *s*, 6.36 *s*
9	4.05 *d* (8) (Ha)	4.71 *d* (9.0) (Ha)	4.12 *d* (8.4) (Ha)	4.06 *d* (9.0), 4.19 *d* (9.0)
	4.88 *d* (8) (Hb)	4.32 *d* (9.0) (Hb)	4.97 *d* (8.4) (Hb)	4.53 *d* (9.0), 4.24 *d* (9.0)
10	1.41 *s*	1.24 *s*	1.53 *s*	1.28 *s*, 1.46 *s*
11	2.69 *tq* (7, 7)	2.85 sep (6.8)		
12	1.73 *ddq* (7, 7, 14)	1.28 *d* (6.8)	7.86 *br d* (8.0)	7.90 *m* × 2
	1.63 *ddq* (7, 7, 14)			
13	0.97 *t* (7)	1.29 *d* (6.8)	7.52 *t* (8.0)	7.47 *m* × 2
14	1.25 *d* (7)		7.62 *br d* (8.0)	7.52 *m* × 2
15			7.52 *t* (8.0)	7.47 *m* × 2
16			7.86 *br d* (8.0)	7.90 *m* × 2

Coupling constants (in Hz) in parentheses.

phosphate and polyketide. The terminal acyl parts probably come from the individual amino acids or benzoic acid. The oxidation and cyclization of an intermediate polyketone A may give hyperolactone D and its acyl analogues which will be oxidized and etherized to give hyperolactones as indicated in Scheme 1.

EXPERIMENTAL

Mps: uncorr.; MS: direct inlet, 70 eV; ^{1}H and ^{13}C NMR: 270 and 68 MHz, respectively, $CDCl_3$, TMS as int. standard. HPLC was performed on a silica gel column (Lober Si-60 eluted with hexane–EtOAc), an ODS column (LiChrosorb RP-18, 7 μm, 10 × 250 mm, eluted with MeOH) and on the gel permeation column (Asahipak GS310, 7.5 × 500 mm, eluted with MeOH) using UV detection.

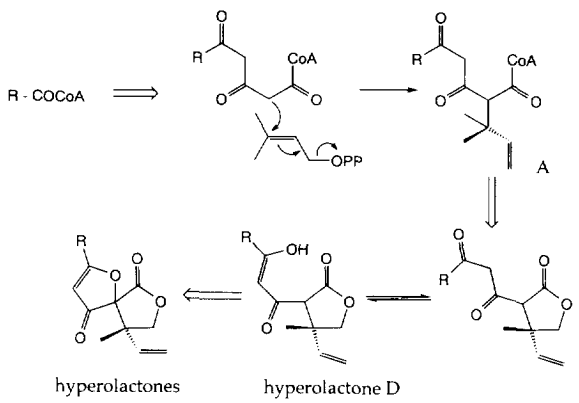
Plant material. The plant material (leaves and stems) of *H. chinense* was collected in the campus of Tokyo University of Agriculture and Technology, in June 1988 and 1992.

Extraction and isolation. Fresh leaves and stems (133 g) were extracted with MeOH at room temp. for 14 days, the extract was concd *in vacuo* and the residue partitioned

Table 2. ^{13}C NMR spectral data of four hyperolactones

C	Hyperolactone A	Hyperolactone B	Hyperolactone C	Hyperolactone D
1	118.8 (CH_2)	116.0 (CH_2)	119.2 (CH_2)	115.3, 116.6 (CH_2)
2	134.2 (CH)	136.7 (CH)	134.2 (CH)	140.7 × 2 (CH)
3	48.3	48.4	48.9	46.9 × 2
4	92.4	91.8	93.1	58.6, 61.0 (CH)
5	197.2	197.5[a]	196.6	187.2, 187.8[c]
6	102.0 (CH)	101.3 (CH)	100.3 (CH)	97.9, 98.0 (CH)
7	200.1	201.3[a]	187.3	184.6, 184.8[c]
8	167.9	168.1	168.1	172.8, 173.2
9	73.9 (CH_2)	73.2 (CH_2)	74.2 (CH_2)	76.4 × 2 (CH_2)
10	19.2 (Me)	15.4 (Me)	19.5 (Me)	18.8, 23.9 (Me)
11	37.0 (CH)	30.4 (CH)	127.7	134.2 × 2
12	27.0 (CH_2)	19.4 (Me)[b]	127.4 (CH)	127.4 × 2 (CH)
13	10.9 (Me)	19.7 (Me)	129.0 (CH)	128.8 × 2 (CH)
14	16.9 (Me)		133.6 (CH)	133.0 × 2 (CH)
15			129.0 (CH)	128.8 × 2 (CH)
16			127.4 (CH)	127.4 × 2 (CH)

δ (DEPT)
[a–c] Signals may be exchangeable.



Scheme 1. Biosynthesis of hyperolactones.

between EtOAc and H_2O. The EtOAc soluble portion was separated into several fractions by silica gel CC eluting with hexane–EtOAc (8:1). The less polar fraction was further separated by HPLC on an ODS column followed by a LoberA Si-60 column eluted with hexane–EtOAc to give **1** (133 mg), **2** (2 mg) and **3** (100 mg). The polar fraction was purified by HPLC on an ODS column eluted with MeOH followed by an Asahipak GS310 column eluted with MeOH to give **4** (7 mg).

Hyperolactone A (**1**). Crystals, mp 57°; $[\alpha]_D$ − 228.9° (MeOH; *c* 0.13). HR-MS $[M]^+$ at *m/z* 250.1195; calcd for $C_{14}H_{18}O_4$ $[M]^+$ 250.1205. MS *m/z*: 250 $[M]^+$ 193, 177, 167, 153, 95, 81. IR ν_{max}^{KBr} cm^{-1}: 3100, 1796, 1701, 1647, 1615, 1596, 1585, 1010, 981. UV λ_{max}^{EtOH} nm (ε): 209 (3.5 × 10^3), 267 (9.4 × 10^3).

Hyperolactone B (**2**). Crystals, mp 54°; $[\alpha]_D$ + 411.1 (EtOH; *c* 0.018). HR-MS $[M]^+$ *m/z* 236.1044; calcd for $C_{13}H_{16}O_4$ $[M]^+$ 236.1049. MS *m/z*: 236 $[M]^+$ 193, 177, 153, 95, 81. IR ν_{max}^{KBr} cm^{-1}: 1795, 1690, 1640, 1595. UV λ_{max}^{EtOH} nm (ε): 203 (1.4 × 10^4), 267 (2.0 × 10^4).

Hyperolactone C (**3**). Crystals, mp 104°; $[\alpha]_D$ − 356.0° (EtOH; *c* 0.02). HR-MS $[M]^+$ at *m/z* 270.0887; calcd for $C_{16}H_{14}O_4$ $[M]^+$ 270.0892. MS *m/z*: 270 $[M]^+$ 252, 225, 211, 188, 187, 173, 147, 105, 102, 77. IR ν_{max}^{KBr} cm^{-1}: 3100, 3070, 2925, 1780, 1710, 1645, 1590, 1560. UV λ_{max}^{EtOH} nm (ε): 254 (1.4 × 10^4), 307 (2.6 × 10^4).

Hyperolactone D (**4**). Liquid, $[\alpha]_D$ + 54.6° ($CHCl_3$; *c* 0.13); HR-MS $[M]^+$ at *m/z* 272.1026; calcd for $C_{16}H_{16}O_4$ $[M]^+$ 272.1049. MS *m/z*: 272 $[M]^+$ 187, 165, 138, 105, 91, 77. IR ν_{max}^{Neat} cm^{-1}: 3400 (br), 1770, 1600. UV λ_{max}^{EtOH} nm (ε): 220 (6.0 × 10^3), 252 (6.9 × 10^3), 320 (1.8 × 10^4).

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