by CC on silica gel (30 g) and elution with C_6H_6–EtOAc (3:1, 2:1). Appropriate column fractions were separated by prep. TLC using silica gel and C_6H_6–EtOAc (3:1). Subsequent purification on silica gel (20 g) and recrystallization from the same solvent mixture yielded 23 mg of pure **1**.

Compound **1**. Thin needles with blue fluorescence (360 nm). Mp 262–263° (C_6H_6–EtOAc 3:1). $C_{15}H_{10}O_4$ requires $[M]^+$ 254.0579; found 254.0585. EI-MS: *m/z* (rel. int.): 254 $[M]^+$ (100), 225 $[M-CHO]^+$ (90), 139 (40); CI-MS: 255 $[MH]^+$; UV λ_{max}^{MeOH} nm: 230, 257, 266, 289sh, 300, 315, 330sh, 345; IR ν_{max}^{Nujol} cm^{-1}: 1725 (conjugated ester); ^1H NMR (DMSO-d_6, ppm): 3.493 (2H, *t*, $J=6.1$ Hz, CH_2-4), 4.678 (2H, *t*, $J=6.1$ Hz, CH_2-3), 5.851 (2H, *d*, $J=1.1$ Hz, CH_2-6), 7.875 (1H, *dd*, $J=8.5$, 7.3 Hz, H-10), 7.964 (1H, *br s*, H-5), 8.376 (1H, *dd*, $J=7.3$, 1.1 Hz, H-9), 8.517 (1H, *dd*, $J=8.5$, 1.1 Hz, H-11); ^{13}C NMR (DMSO-d_6, ppm, multiplets from DEPT): 23.06 (*t*, C-4), 66.20 (*t*, C-3), 68.73 (*t*, C-6), 120.23 (*d*, C-10), 127.30 (*d*, C-5), 130.29 (*d*, C-9), 130.64 (*d*, C-11), 120.52 *s*, 122.32 *s*, 126.65 *s*, 128.33 *s*, 129.61 *s*, 138.04 *s* (C-4a, C-5a, C-5b, C-8a, C-11a, C-11b), 162.49 *s* and 163.61 *s* (C-1 and C-8).

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ISOSALVIANOLIC ACID C, A DEPSIDE POSSESSING A DIBENZOOXEPIN SKELETON

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Key Word Index—*Salvia chinensis*; Labiatae; depsides; isosalvianolic acid C.

Abstract—Isosalvianolic acid C, a new depside possessing a dibenzooxepin skeleton, was isolated from the aqueous extract of *Salvia chinensis* along with salvianolic acid B and D, lithospermic acid, rosmarinic acid, (*R*-(+)-β-(3,4-dihydroxyphenyl)lactic acid, caffeic acid and protocatechualdehyde.

INTRODUCTION

Salvia chinensis Benth. is a herbal medicinal plant distributed in the southern part of the Yangtze River in China. It is used in Chinese folk medicine for the treatment of hepatitis, nephritis, dysmenorrhea and several kinds of cancer [1]. No chemical investigation of this plant has been reported previously. During our studies on the biologically active components of the traditional Chinese medicine Danshen (*Salvia miltiorrhiza*), we isolated several depsides [2–4], some of which possessed significant antiplatelet [5] and anti-oxidant activities [6]. These observations prompted us to extend our studies to other species of the genus *Salvia*. Chemical studies on the aqueous extract of *S. chinensis* led to the isolation of five depsides, among which one is a new compound named isosalvianolic acid C (**1a**), together with two phenolic acids and protocatechualdehyde.

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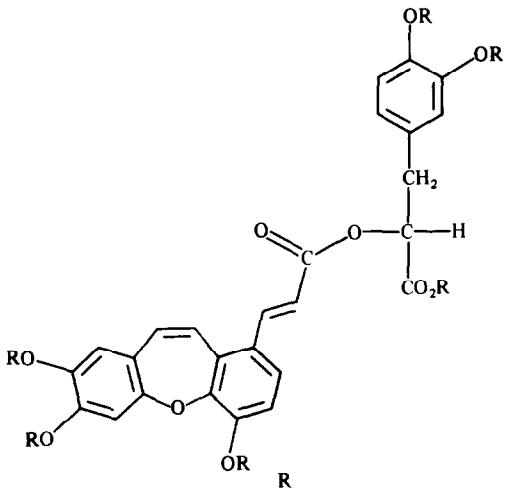
RESULTS AND DISCUSSION

Systematic isolation of the aqueous extract of *S. chinensis* by Sephadex LH-20 CC, silica gel LPLC and preparative TLC yielded a new depside named isosalvianolic acid C (**1a**), along with four known depsides, salvianolic acid B [3] and D [4], lithospermic acid and rosmarinic acid, two phenolic acids, *R*-(+)-β-(3,4-dihydroxyphenyl)lactic acid and caffeic acid, and protocatechualdehyde.

Isosalvianolic acid C (**1a**) was recognized as a phenolic acid from a positive test with ferric ferricyanide and bromocresol green. UV maxima at 202, 222, 288, 326 and 340 nm suggested the presence of a highly conjugated system. Its FD mass spectrum displayed $[M]^+$ at *m/z* 492, similar to that of salvianolic acid C [3].

The HR mass spectrum of the methylated compound **1b** showed $[M]^+$ at *m/z* 576.2000 (calc. for $C_{32}H_{32}O_{10}$, 576.1996) and diagnostic fragments of a β-(3,4-dimethoxyphenyl)-lactic ester (*m/z* 222, 191, 181, 163) [2].



1a R = H
1b R = Me

Hydrolysis of **1b** with 10% KOH–methanol yielded *R*-(+)-β-(3,4-dimethoxyphenyl)lactic acid [2] and a yellowish crystalline compound **1c** with $[M]^+$ at *m/z* 354. Its ^1H and ^{13}C NMR spectra revealed the presence of 16 olefinic carbons, eight olefinic protons, three methoxyls and one carboxyl group (Table 1), giving a composition $C_{20}H_{18}O_5$ of 338. The molecular ion of **1c** indicated the presence of an additional oxygen, linked between two olefinic carbons, thus **1c** should have a molecular formula of $C_{20}H_{18}O_6$. Consideration of the ^1H and ^{13}C NMR data indicated that the 12 degrees of unsaturation inherent in the molecule consisted of one carboxylic carbonyl, eight olefinic groups and a tricyclic carbon skeleton. A dibenzooxepin skeleton for **1c** was deduced from the fact that the two olefinic proton signals at δ6.94 and 7.00 possessed a coupling constant of $J = 11.5$ Hz similar to that of pacharin, a dibenzooxepin derivative isolated from the heartwood of *Bauhinia racemosa* [7]. A pair of singlets at δ6.80 and 6.90 and a pair of doublets with $J = 8$ at δ7.11 and 7.53 indicated the presence of two *para* protons and two *ortho* protons in the two separated benzo groups. The chemical structure of **1c** was finally verified by H,C-COLOC analysis as depicted in Fig. 1. Isosalvianolic acid C is thus a depside having structure **1a**. Following the isolation of salvianolic acid G from *S. miltiorrhiza* [8], this is the second dibenzooxepin derivative isolated from the genus *Salvia*.

EXPERIMENTAL

General. Sephadex LH-20 CC, solvent MeOH; LPLC, 300 g silica gel, sample 4 g, solvent **A**, $CHCl_3$–MeOH–HCO_2H (85:15:1) 2 l, **B**, $CHCl_3$–MeOH (17:3) 1.5 l, **C**, (4:1) 800 ml, **D**, (1:1) 800 ml; TLC, silica gel-GF_{254}, solvent $CHCl_3$–MeOH–HCO_2H (85:15:1). Mps: uncorr. ^1H and ^{13}C NMR (90 or 400 MHz) with TMS as int. standard.

Plant material. The whole plants of *S. chinensis* were collected in Jiangxi province, China, and identified by Prof. Zhang Haidao of the Jiangxi Institute of Materia Medica. A voucher specimen is deposited in the Institute of Materia Medica in Beijing.

Extraction and isolation of the depsides. The dried plant material (4.25 kg) was extracted with 95% EtOH and the residue boiled with H_2O. The aq. extract was concd under red. pres. and EtOH added to the concentrate until the EtOH content was 70%. After filtering, the filtrate was concd to 2 l and successively extracted with $CHCl_3$ and EtOAc. Evapn of the EtOAc extract yielded 4.6 g of fr. 1. The aq. portion was acidified with 10% HCl to pH 3–4 and successively extracted with EtOAc and *n*-BuOH. After removal of the solvent, 38.5 g of fr. 2 and 23.94 g of fr. 3 were respectively obtained from the EtOAc and *n*-BuOH extracts. Chromatography of fr. 1 (3.9 g) over Sephadex LH-20 afforded six fractions. Purification of frs 1–2 and 1–3 by prep. TLC yielded 99 mg of protocatechualdehyde and 40 mg of caffeic acid. Fr. 2 (34 g) was sepd by LPLC and 12 frs were collected. Rechromatography over Sephadex LH-20 yielded rosmarinic acid (347 mg) from fr. 2–4, isosalvianolic acid C (**1a**) (180 mg) from fr. 2–5, and salvianolic acid D (30 mg) and lithospermic acid (167 mg) from fr. 2–6. Further fractionation of fr. 2–7 by prep. TLC furnished 1.07 g of salvianolic acid B. Separation of fr. 3 (4 g) by Sephadex LH-20 CC, followed by prep. TLC led to the isolation of *R*-(+)-β-(3,4-dihydroxyphenyl)lactic acid (52 mg).

Isosalvianolic acid C (**1a**). Amorphous yellowish compound, $[\alpha]_D^{12} + 39°$ (EtOH; *c* 0.06). UV λ_{max}^{EtOH} nm (log ε): 202 (4.77), 222 (4.46 sh), 288 (4.21), 326 (4.30), 340 (4.24); FD-MS *m/z*: 492 $[M]^+$ ^1H NMR (Me_2CO-d_6): δ5.20 (1H, *dd*, $J = 6$, 8 Hz), 6.28 (1H, *d*, $J = 16$ Hz), 7.92 (1H, *d*, $J = 16$ Hz), 6.72–7.41 (9H, *m*).

Methylation of isosalvianolic acid C. Dry K_2CO_3 (200 mg) was suspended in a soln of **1a** (80 mg) in dry Me_2CO (10 ml) under an atmosphere of N_2. Me_2SO_4 (1.5 ml) was added, and after stirring for 6 hr a second portion (0.5 ml) added and stirring was continued for 26 hr. The mixture was filtered and concd under red. pres. The residue was purified by silica gel VLC [9] using a mixture of petrol and EtOAc with increasing polarity. Methylated **1b** (72 mg) was obtained as a yellowish gum. $[\alpha]_D^{17} + 21°$ (EtOH; *c* 0.06). UV λ_{max}^{EtOH} nm (log ε): 203 (4.72), 232 (4.40 sh), 288 (4.07), 326 (4.22), 340 (4.15); HRMS *m/z* (rel. int.): 576.2000 $[M]^+$ (100) ($C_{32}H_{32}O_6$ requires 576.1996), 354 (39), 337 (24), 309 (72),

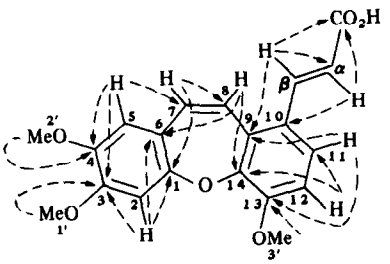


Fig. 1. H, C-COLOC of **1c**

Table 1. ^{13}C and ^1H NMR spectral data of compound **1c** (DMSO-d_6)

C	^{13}C	^1H	C	^{13}C
2	105.3	6.80 (1H, *s*)	1	150.7
5	111.6	6.90 (1H, *s*)	3	146.0
7	131.2	6.94 (1H, *d*, $J = 11.5$)	4	150.4
8	124.4	7.00 (1H, *d*, $J = 11.5$)	6	121.9
11	124.2	7.53 (1H, *d*, $J = 8$)	9	130.1
12	112.7	7.11 (1H, *d*, $J = 8$)	10	124.5
1′	55.9	3.72 (3H, *s*)	13	152.5
2′	55.7	3.77 (3H, *s*)	14	145.7
3′	56.1	3.91 (3H, *s*)	C=O	167.2
α	119.6	6.30 (1H, *d*, $J = 16$)		
β	134.0	7.80 (1H, *d*, $J = 16$)		

278 (37), 222 (98), 191 (42), 181 (19), 163 (13), 151 (77); ^{1}H NMR ($CDCl_3$): δ3.11 (1H, *d*, J = 6 Hz), 3.12 (1H, *d*, J = 8 Hz), 5.28 (1H, *dd*, J = 6, 8 Hz) 3.72, 3.86, 3.92 (each 3H, *s*) 3.84 (9H, *s*), 6.20, 7.91 (each 1H, *d*, J = 16 Hz), 6.61–7.32 (9H, *m*).

Hydrolysis of compound **1b**. Compound **1b** (40 mg) was refluxed with 5 ml 10% KOH–MeOH for 1 hr. After acidification and work-up with EtOAc and H_2O, the organic layer was concd yielding 19 mg of yellow crystals **1c**. The mother liquor was purified by TLC using C_6H_6–EtOH–HCO_2H (80:20:1) as solvent, 5 mg of **1c** along with *R*-(+)-β-(3, 4-dimethoxyphenyl)lactic acid were obtained.

Compound **1c**. Yellow crystals, mp 178–180°. EI-MS *m/z* (rel. int.): 354 ([M]$^+$, 100), 339 (28), 309 (55), 278 (75).

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CHROMENES FROM *ARNICA SACHALINENSIS* AND *A. AMPLEXICAULIS*

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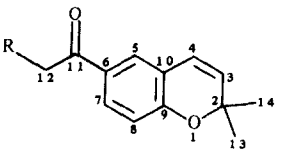
Key Word Index—*Arnica sachalinensis*; *A. amplexicaulis*; Asteraceae; chromenes (benzopyrans); 12-acetoxy-demethoxyencecalin; 12-isobutyryloxy-demethoxyencecalin.

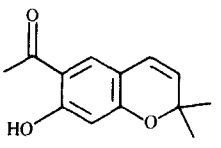
Abstract—Flowerheads of *Arnica sachalinensis* yielded, in addition to the known chromenes acetovanillochromene, demethylencecalin and demethoxyencecalin, the new compounds 12-acetoxy-, 12-isobutyryloxy- and 12-isovaleryloxy-demethoxyencecalin. Demethoxy-and demethylencecalin are also constituents of *A. amplexicaulis*. The assignment of the ^{13}C NMR signals of demethoxyencecalin is revised by 2D–^{13}C, ^{1}H NMR experiments. Chemotaxonomic aspects are discussed.

INTRODUCTION

Chromenes (benzopyrans) and benzofurans are prominent natural products of many genera of the Asteraceae and have become useful taxonomic markers within this family [1–3]. In a previous paper [4], we reported on the isolation of demethoxyencecalin (**1**) from the flowers of *Arnica sachalinensis* (Regl.) A. Gray, which was the first chromene found in the genus *Arnica*. The tribal position of the genus *Arnica*, traditionally within the tribe Senecioneae, has been the subject of considerable discussion [5, 6]. On morphological, serological and chemical grounds, it has been argued that *Arnica* is more related to the Heliantheae than to the Senecioneae, thus Robinson [7] includes *Arnica* in this tribe, subtribe Chaenactidinae. In Bohlmann's opinion, however, *Arnica* has more resemblance to the closely related subtribe Gaillardiinae



	R
1	H
2	OAc
3	OiBut
4	OiVal or O-2-MeBut



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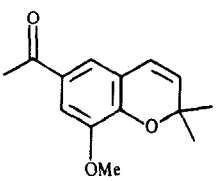

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