Short Communications

Structure revision and cytotoxicity of the germacranolide, stizolicin, from *Stizolophus balsamitus* (Asteraceae)

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Summary. The structure of the cytotoxic sesquiterpene lactone, stizolicin, reisolated from *Stizolophus balsamitus (Centaurea b.)* was revised to a *trans, trans* germacranolide (**1**) on the basis of simultaneous application of lanthanide shift reagent and NOE in the NMR.

Key words. Stizolicin; germacranolide; *Stizolophus balsamitus;* Asteraceae; structure revision.

Stizolophus balsamitus Cass. *ex* Takht. (*Centaurea b.* Lam.) (Asteraceae) is endemic to the Caspian Sea area; 850 g of the plant[3] was extracted with ethanol and the ethanol residue was partitioned (chloroform-water). The chloroform residue was partitioned (hexane-90% aq. methanol); the 90% methanol was treated with 4% aq. lead acetate, filtered, concentrated to remove methanol, and the remaining aqueous solution was extracted (chloroform). After chromatographic resolution of the chloroform residue on 2 silica gel columns (chloroform-methanol gradients), a fraction eluted in 5% methanol-chloroform yielded crystals from ethanol of compound (**1**): $[\alpha]_D^{25}$ −31° (c 2.19 abs. ethanol), $C_{20}H_{26}O_7$ (found: C, 63.25; H, 6.98; calc.; C, 63.48; H, 6.93), M^+, M/z 378.172.

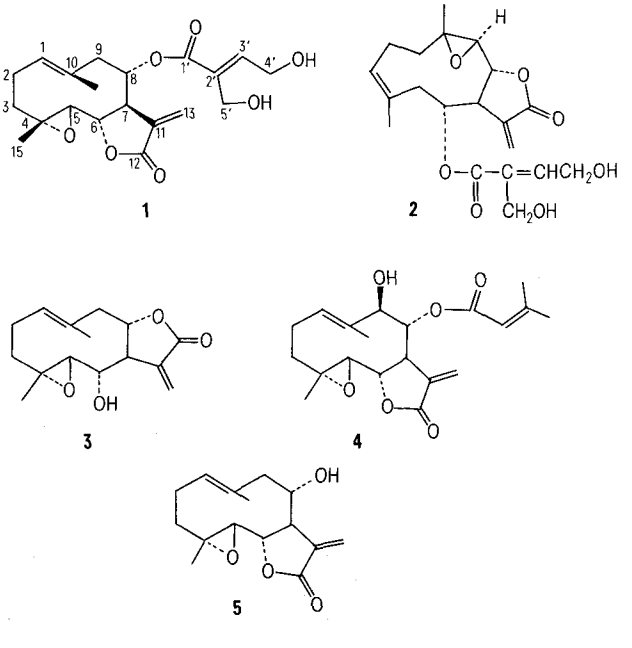
The presence in **1** of an α-methylene-γ-lactone was suggested by the UV (λ_{max}^{MeOH} 208 nm, ε = 21,500), the IR absorption at 1760 cm^{-1}, and the pair of doublets at δ 6.28 and δ 5.68 in the proton NMR (table). A 4′,5′-dihydroxy tiglate ester moiety was apparent in the MS peak m/z 246 ($M^+-C_5H_8O_4$), in the IR (1714 and 3450 cm^{-1}), in the UV extinction coefficient of 21,500, and in the proton NMR resonances at δ 6.97 (t, J = 5.8 Hz, 3′ vinyl H), 4.46 (d, J = 5.8 Hz, 4′-CH_2), and 4.32 (s, 5′-CH_2). These resonances corresponded closely with those of eupaformosanin, the structure of which was established by X-ray crystallography[4]. The 470 MHz NMR of **1** and extensive decoupling experiments made the complete assignment of the proton NMR spectrum possible. The chemical shifts of H-6 and H-8 (δ 4.31 and δ 4.55, respectively) required that these be located at the esterification sites, while the position of H-5 (δ 2.63) and lack of protons on C-4 suggested a 4,5-epoxygermacranolide. Compound (**1**), thus, seemed identical (m.p., proton NMR, IR, UV, $[\alpha]_D$) with stizolicin which had previously been isolated from *S. balsamitus*[5], *S. coronopifolius* (Lam.) Cass.[6], *Centaurea solstitialis* L.[7], and *Saussurea elongata* DC.[8], all members of the Asteraceae tribe Cardueae. Chemotaxonomic considerations, however, caused us to question the previously reported structure of stizolicin as a *cis, cis* germacranolide (**2**) since such compounds are otherwise unknown in this tribe[9].

A lack of nuclear Overhauser effect (NOE) in the NMR at H-1 upon irradiating H-14, together with the small allylic coupling ($J_{1,14}$ = 1 Hz), requires that the C-1,10 double bond be *trans* (or E). The corresponding experiment between H-5 and H-15 produced a positive NOE at H-5, although the interpretation is ambiguous due to overlap of the H-15 and H-3α resonances even at 470 MHz; (the NOE is, in fact, caused by dipole interaction between H-3 and H-5). A similar experiment involving irradiation of H-15 and observation of H-6 was complicated by the H-4′ and H-5′ resonances obscuring the H-6 peak. The addition of 0.2 equivalent of dry $Eu(fod)_3$ shifted the ester protons downfield selectively. By employing sealed and freeze-pump-thaw-deoxygenated tubes, NOE's of 14–16% were ob-

NMR chemical shifts and couplings for stizolicin[a]

Assignment	δ (ppm)		Couplings (Hz)
$CDCl_3$	7.24	s	
H-3	6.97	t (1)	J3′,4′=5.8
H-13b	6.28	d (1)	J13b,7=3.4
H-13a	5.68	d (1)	J13a,7=3.0
H-1	5.29	br dd (1)	J1,2α ≃ 3; J1,2β ≃ 11.5
			J1,14 ≃ 1
H-8	4.55	ddd (1)	J8,7=4.2
			J8,9α=10.9; J8,9β=1.5
H-4′	4.55	d (2)	J4′,3′=5.8
H-5′	4.32	s (2)	
H-6	4.31	dd (1)	J6,5=9.3; J6,7=6.8
H-7	3.29	m (1)	Σ=18–20
H-5	2.63	d (1)	J5,6=9.3
H-9α	2.55	dd ()	J9α,9β=11.9; J9α,8=11.4
H-9β	2.46	dd (1)	J9β,9α=12.0; J9β,8=1.5
H-2β	2.40	obs ddd (1)	J2β,2α=12.6; J2β,1=11.5
			J2β,3α=5.9; J2β,3β=5.9
H-2α	2.27	obs m (1)	J2α,2β=12.5; J2α,1=3
			J2α,3α ≃ 6.0; J2α,3β ≃ 0
−OH	2.26	D_2O exchangeable	
H-3β	2.16	ddd (1)	J3β,3α=13.0; J3β,2β=5.7
H-14	1.80	br s (3)	J14,1 ≃ 1
−OH	1.58	D_2O exchangeable	
H-15	1.27	s (3)	
H-3α	1.23	obs dd(1)	J3α,3β=12.8; J3α,2α ≃ 6.4
			J3α,2β ≃ 6.4

[a] Reported couplings are from 80, 360, or 470 MHz spectra with sample dissolved in $CDCl_3$, d-6 acetone or d-5 pyridine as needed. Chemical shifts are from spectra run in $CDCl_3$.



served at H-6; H-15 and H-6 are clearly on the same molecular face. The observed $J_{5,6} = 9.6$ Hz requires that H-5 and H-6 be *trans* to one another; this completed the proof that stizolicin is, indeed, a *trans,trans* germacranolide. A complex analysis of the coupling constants for H-3, H-2, and H-1 also demonstrated that both methyl groups are oriented in the energetically-favored β-orientations[10].

Trans lactone closure follows from the observed $J_{7,13} > 3.0$[11,12] and closure to C-6 is evident in the negative Cotton Effect ($\theta = -3780$ at λ_{max}^{MeOH} 250 nm) for the n→π* transition[13]. The stereochemistry at C-8 can be assigned based on chemical shift analysis. The β-esters have H-8 resonating around δ 5.7 due to location within the plane of the C-11,13 double bond, while α-esters show H-8 near δ 4.5[14]. Stizolicin has an α-ester in consonance with all other esterified sesquiterpene lactones from this tribe. The structural proof of stizolicin was concluded by hydrolysis in sodium hydroxide-aqueous dioxane to give the rearranged lactone, isospiciformin (**3**). The NMR of this product was identical with that obtained from an authentic sample[15] prepared from desacetyllaurenobiolide epoxidation.

Several other compounds are also known from *S. balsamitus*. The related balsamin (**4**)[16] and stizolin (**5**)[17] are spectroscopically similar to stizolicin, and, thus, are probably also *trans,trans* and not *cis,cis* germacranolides. In addition, the flavonoid 5-O-β-D-glucosyl-3-O-methylquercitin[18] and an alkaloid, stizolophin ($C_{15}H_{23}NO_5$), have been isolated[19].

Stizolicin (NSC 301458) showed cytotoxicity ($LD_{50} = 9.4 \times 10^{-1}$ µg/ml and 4.7 µg/ml in the P388 and KB tumor cell cultures, respectively) and marginal in vivo antitumor activity against P388 murine leukemia (T/C = 123% at 16 mg/kg). A similar compound, eupatoriopicrin, lacking an epoxy and with the same ester beta, has shown slightly better activity in P388 (T/C = 140% at 30 mg/kg)[20].

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3 Epigeal portions collected in June 1978 from Karaj, 40 km west of Tehran, Iran, and shade dried. A voucher specimen (No. 86) is on deposit in the herbarium of the Department of Pharmacognosy, University of Tehran, as *Centaurea balsamita* Lam.
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12-Hydroxy-*E*-γ-bisabolene, a new sesquiterpene alcohol from a Caribbean sea whip of the genus *Pseudopterogorgia* (Gorgonacea, Cnidaria)

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Summary. A new sesquiterpene alcohol, 12-hydroxy-*E*-γ-bisabolene, is reported from an undescribed Caribbean sea whip of the genus *Pseudopterogorgia*. The structure of this new alcohol was established based upon spectral analyses and through chemical interconversions.

Key words. Caribbean sea whip; sesquiterpene alcohol; *Pseudopterogorgia*; 12-hydroxy-*E*-γ-bisabolene.

Sea whips (gorgonians) of the genus *Pseudopterogorgia* are particularly abundant in the Caribbean Sea and several new terpenoid metabolites have been recently isolated from this source[3,4]. In our studies of these chemically rich marine invertebrates we have found the secondary metabolite composition to be consistent within discrete species, and hence secondary metabolites appear to be potentially useful taxonomic markers. Two of our collections of *Pseudopterogorgia* (voucher specimens: F-24, Belize, 1979, Florida Keys, 1980) were distinct in their physical features from other common species encountered, but anatomical investigations (spicule analyses) placed this animal as closely related to the abundant Caribbean sea whip *P. acerosa*[5]. In previous chemical studies we showed that *P. acerosa* produces the diterpenoid molecule pseudopterolide (**1**). In this paper we wish to show that this gorgonian (F-24) produces exclusively the new sesquiterpene alcohol **2**, identified here as 12-hydroxy-*E*-γ-bisabolene.

Alcohol **2** was isolated by repetitive chromatography as 20% of the organic extracts of *Pseudopterogorgia* species conforming morphologically to voucher F-24. High-resolution mass spectrometry and ^{13}C NMR data (table) confirmed a molecular formula of $C_{15}H_{24}O$ for the compound[6]. Infrared absorption at 3350 cm^{-1}, coupled with appropriate ^{13}C NMR bands showed that the oxygen atom in **2** was in the form of a primary alco-