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# Cardiobutanolide, a styryllactone from *Goniothalamus cardiopetalus*

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**Abstract**

A styryllactone namely cardiobutanolide was isolated from the stem bark of *Goniothalamus cardiopetalus* together with four known styryllactones goniothalamin, goniodiol, goniofufurone, goniofupyrone and known acetogenins squamocin and an epimeric mixture of goniodonin and 34-*epi*-goniodonin. The structure of the new compound was elucidated on the basis of 1D and 2D NMR experiments and mass spectroscopic techniques.


*Keywords:* Annonaceae; *Goniothalamus cardiopetalus*; Styryllactones; Acetogenins

## 1. Introduction

In our earlier report on the phytochemical investigation of *Goniothalamus cardiopetalus* (Annonaceae), we have reported the isolation and characterization of a novel styryllactone namely cardiopetalolactone together with two known lactones altholactone and goniopypyrone (Hisham et al., 2000). As an extension to the earlier study, we have examined the EtOAc soluble fraction of the EtOH extract and isolated five more styryllactones and two acetogenins of which one of the styryllactones was found to be new. The details of the work is reported here.

## 2. Results and discussion

The concentrated EtOH extract of the stem bark was partitioned between hexane/$H_2O$ and EtOAc/$H_2O$ as described previously. After the preliminary TLC analysis, the EtOAc soluble portion was column chromatographed over a silica column which was eluted with solvents of increasing polarity. Eight styryllactones (**1**–**8**) and two acetogenins (**9, 10**) were eluted and which were further purified by Prep. TLC. Compounds **2**–**4** were found to be the previously isolated cardiopetalolactone, altholactone and goniopypyrone respectively. On the basis of $^1$H and $^{13}$C NMR data, compound **1** was identified as goniothalamin (Talapatra et al., 1985), **5** as goniodiol (Fang et al., 1991a,b), **6** as goniofufurone (Fang et al., 1990), **7** as goniofupyrone (Fang et al., 1991a,b), **9** as squamocin (Fujimoto et al., 1988) and **10** as an epimeric mixture of goniodonin and 34-*epi*-goniodonin (Jiang et al., 1997).

Compound **8**, a new styryllactone, was obtained as white crystals from the polar fractions and was given a trivial name cardiobutanolide. Its molecular formula, $C_{13}H_{16}O_6$ was obtained from high resolution FAB mass spectrum which showed $[M+H]^+$ ion at $m/z$ 269.1073 corresponding to the formula $C_{13}H_{17}O_6$ (calc 269.1025). The IR spectrum showed bands at 3432, 1731 and 1600 cm$^{-1}$ for hydroxyl, γ-lactone and phenyl groups respectively. The presence of a phenyl group was evident from signals at $\delta$ 7.24 (1H, *t*, $J=7.7$ Hz), 7.32 (2H, *t*, $J=7.7$ Hz) and 7.45 (2H, *d*, $J=7.7$ Hz) in the $^1$H NMR spectrum and signals at $\delta$ 127.95 (*d*, CH×2) 127.84 (*d*), 128.60 (*d*, CH×2) and 144.26 (*s*) in the $^{13}$C NMR spectrum. The presence of a γ- lactone unit was primarily assigned on the basis of a lactone carbonyl carbon shift at $\delta$ 176.05 and an oxymethine carbon shift at $\delta$ 86.57. The presence of four secondary hydroxyl groups was evident from the following spectroscopic information.

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The FAB mass spectrum showed intense ions at $m/z$ 251, 233, 215 and 197 formed by the expulsion of one, two, three and four molecules of water respectively from the $[M+H]^+$ ion. In the $^{13}C$ NMR spectrum, the signals at $\delta$ 75.59 (*d*), 74.15 (*d*), 70.34 (*d*) and 68.68 (*d*) were obviously due the carbinol methine carbons. The $^1H$ NMR spectrum showed four oxymethine proton signals at $\delta$ 4.79 (*d*, J = 7.8 Hz), 4.61 (*dd*, *J* = 5.1, 3.6 Hz), 4.38 (*dd*, J = 7.8, 1.7 Hz) and 3.91 (*dd*, *J* = 7.8, 1.7 Hz) in addition to the signal at $\delta$ 4.55 (1H, *dd*, *J* = 7.8, 3.6 Hz) assignable to H-4 of the lactone ring which correlated with the methine carbon at $\delta$ 86.57 in the HMQC spectrum. In addition, the $^1H$ NMR spectrum exhibited two one-proton signals at $\delta$ 2.85 (*dd*, *J* = 17.3, 5.1 Hz) and 2.37 (*dd*, J = 17.3, 1.0 Hz) which were assignable for the diastereotopic methylene protons adjacent to the carbonyl group in the lactone ring. The multiplicity of these signals as doublet of doublets indicated the presence of a neighboring proton thereby suggesting one of the carbinol methine groups was adjacent to the methylene group. The planar structure of **8** was assigned on the basis of $^1H$–$^1H$ COSY and HMQC experiments and the data obtained are summarized in Table 1. The entire sequence of $^1H$–$^1H$ spin– spin coupling connectivities starting from C-2 to C-7 were observed in the COSY spectrum which allowed to place all the functional groups as shown in Scheme 2 and the HMQC data led to the assignment of all the carbon shifts in the molecule.

The assignment of stereochemistry of **8** was made on the basis of its $^1H$ NMR chemical shift and coupling constant data comparison with those of two synthetic model compounds prepared as follows.

Enantiospecific syntheses of styryllactones such as goniofufurone, and 7-*epi*-goniofufurone were recently accomplished from commercially available and inexpensive D-glycero-D-gulo-heptono-γ-lactone (Shing et al., 1995). In the case of enantiospecific synthesis of goniofufurone (**6**) and 7-*epi*-goniofufurone, two immediate synthetic precursors namely 7-C-phenyl-D-*gluco*-hept-2-enono-γ- lactone (**11**) and 7-C-phenyl-L-*ido*-hept-2-enono-γ-lactone (**12**) were prepared from the starting material. It was reported that compounds **11** and **12** readily underwent Michael-type cyclization induced by 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) to obtain natural goniofufurone and 7-*epi*-goniofufurone respectively. The structure of compound **8** is very close to compounds **11** and **12** except with a difference that the double bond in the lactone rings in **11** and **12** was replaced by a hydroxyl group in compound **8** at C-3 position. The $^1H$ NMR data reported for these compounds are given in Table 1. The chemical shift value, multiplicity and coupling constant data for H-7, H-6 and H-5 in **8** were virtually identical to those analogous protons in model compound **11** and were different from those of its C-7 epimer **12** thereby suggesting an identical H-7/H-6 *erythro* and H-6/H-5 *threo* relative stereochemistry both in **8** and **11**. Compounds **11** and **12** were assigned with absolute configuration of 7*R*, 6*R,* 5*S* (described as 7*R,* 6*S,* 5*S* incorrectly in the paper) and 7*S,* 6*R,* 5*S* (described as 7*S,* 6*S,* 5*S* incorrectly in the paper), respectively, in a recent study (Su et al., 2001) and therefore compound **8** must have 7*R**, 6*R**, 5*S** stereochemistry in comparison with that in compound **11**. The stereochemical relationship between H-5 and H-4 in **8** could not be decided from the above NMR data, but was assumed to be *erythro* as shown in the structure from the biogenetic considerations of a series of natural styryllactones such as goniotriol and goniofufurone. The large *J* value (7.8 Hz) for H-5/H-4 protons appears to indicate an anti parallel relationship for H-5 and H-4

Table 1
$^1H$ and $^{13}C$ NMR data[a] for compound **8** (400/100 MHz. $d_6$-acetone) and $^1H$ NMR data for model compounds[b] **11** and **12** (250 MHz, $d_6$-acetone)

| Atom no. | **8** | | **11** | **12** |
|---|---|---|---|---|
| | $\delta^{13}C$ | $\delta^1H$ (mult., *J* = Hz) | $\delta^1H$ (mult., *J* = Hz) | $\delta^1H$ (mult., *J* = Hz) |
| 1 | 176.05 *s* | – | – | – |
| 2 | 40.37 *t* | 2.37 (*dd*,17.3, 1.0)<br>2.85 (*dd*, 17.3, 5.1) | 6.15 (*dd*, 5.8, 2.1) | 5.96 (*dd*, 5.8, 2.1) |
| 3 | 68.68 *d* | 4.61 (*dd*, 5.1, 3.6) | 7.82 (*dd*, 5.8, 1.5) | 7.64 (*dd*, 5.8, 2.1) |
| 4 | 86.57 *d* | 4.55 (*dd*, 7.8, 3.6) | 5.27(*ddd,* 5.7, 2.1, 1.5) | 5.10 (*ddd*, 5.8,2.1, 1.5) |
| 5 | 70.34 *d* | 4.38 (*dd*, 7.8, 1.7) | 4.09 (*dd*, 5.7, 2.1) | 3.58 (*dd*, 5.8, 2.9 |
| 6 | 74.15 *d* | 3.91 (*dd*, 7.8, 1.7) | 3.70 (*dd*, 7.9, 2.1) | 3.41 (*dd*, 5.1, 2.9) |
| 7 | 75.59 *d* | 4.79 (*d*, 7.8) | 4.78 (*d*, 7.9) | 4.77 (*d,* 5.1) |
| 8 | 144.26 *s* | – | – | – |
| 9,13 | 127.95 *d* | 7.45 (*d*, 7.7) | 7.24–7.45 (*m*) | 7.29–7.39 (*m*) |
| 10,12 | 128.60 *d* | 7.32 (*t*,7.7) | 7.24–7.45 (*m*) | 7.29–7.39 (*m*) |
| 11 | 127.84 *d* | 7.24 (*t*, 7.7) | 7.24–7.45 (*m*) | 7.29–7.39 (*m*) |

[a] Assignments based on $^1H$–$^1H$ COSY and HMQC.
[b] Data from Shing et al., 1995.

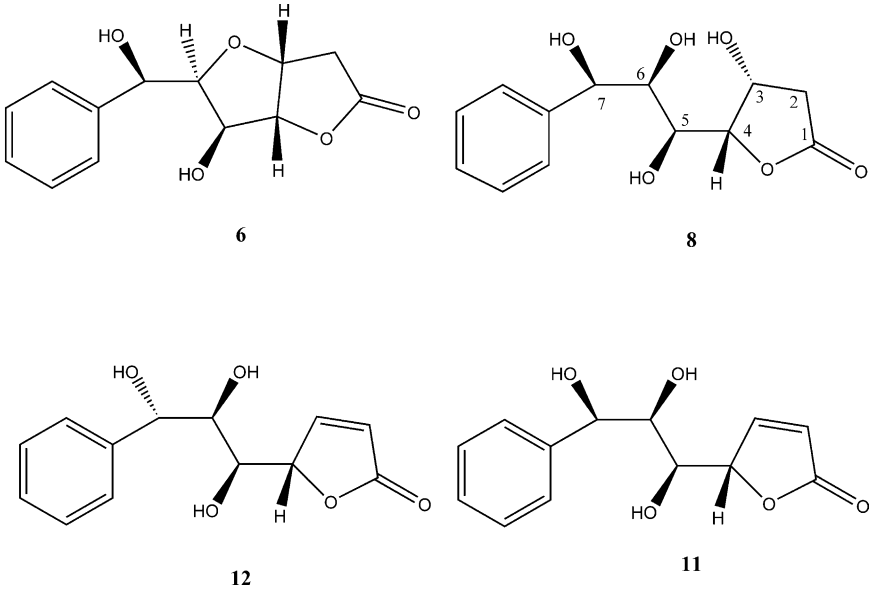
in a preferred conformation as shown in Scheme 2. As the stereochemistry of C-5 is already assigned as *S**, C-4 must have *S** stereochemistry. Finally, the stereochemistry of C-3 hydroxyl group was deduced as *R** on the basis of a smaller coupling constant of 3.6 Hz due to the *cis* configuration of H-4/H-3. Hence, the relative stereochemistry of cardiobutanolide has been assigned as 3*R**, 4*S**, 5*S**, 6*R**, 7*R** as shown in Scheme 1. It is likely that the absolute configuration of cardiobutanolide, 3*R*, 4*S*, 5*S*, 6*R*, 7*R*, is in agreement with that of goniofufurone, by assuming that both compounds could be derived from the same biosynthetic precursor. This was partially supported by a positive molecular rotation value, $[\alpha]_D^{24}+6.4$ for 8 ($[\alpha]_D^{25}+9.0$ for natural form of **6** and $[\alpha]_D^{25}-10.2$ for the enantiomer (Su et al., 2001)). *Goniothalamus cardiopetalus,* resembles *Goniothalamus giganteus* very much in terms of styryllactone contents by elaborating the important styryllactones present in the later plant thus providing another good source of these bioactive natural products.

## 3. Experimental

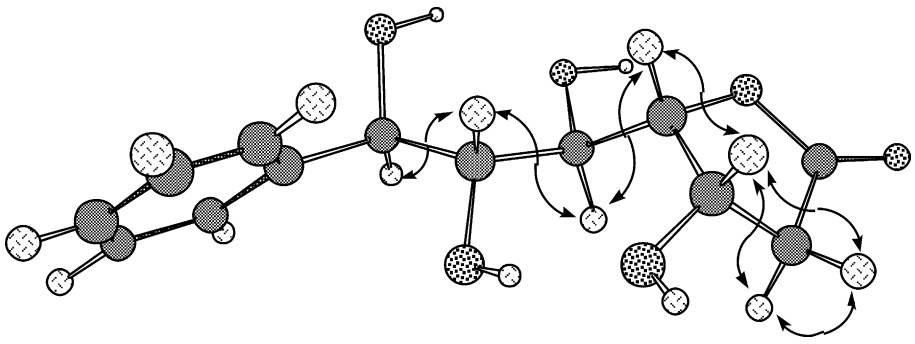
### 3.1. General

The MPs were recorded on a YAZAWA BY-1 micro melting point apparatus and are uncorrected. $^1$H and $^{13}$C NMR spectra were recorded on Jeol Lamda or Brucker Avance 400 MHz spectrometer at 24° C. Tetrmethylsilane (TMS) was used as the internal standard for $^1$H NMR and $d_6$- acetone signal ($\delta=29.80$) or $CDCl_3$ ($\delta=77.0$) was used as a reference for $^{13}$C NMR. All the 1D and 2D acquisition were accomplished with standard Jeol or Brucker pulse programes.

HR–FABMS were performed on a Jeol JMS-AX 505HA spectrometer using glycerol as liquid matrix. Thin-layer chromatography (TLC) was performed on Merck $F_{254}$ silica gel plates (0.25 mm thickness ) and styryllactone were detected by spraying with vanillin-sulfuric acid followed by heating the plates at 100 °C for 5–10 min until the appearance of a spot. Annonaceous




Scheme 1. Structures of styryllactones.



Scheme 2. Perspective 3D representation of structure of **8** showing $^1$H–$^1$H spin–spin coupling connectivities as revealed by COSY spectrum.

acetogenins were detected by spraying with dragendorff reagent to visualize orange yellow spots.

### 3.2. Plant material

*Goniothalamus cardiopetalus* Hook.f. & Thomas was collected from Palaruvi forests in Kerala, India and was authenticated by Dr. Indira Balachandran, Research Officer, Kottakkal Arya Vaidyasala Herbal Gardens, Kottakkal, Kerala, India where a voucher specimen is deposited.

### 3.3. Extraction and isolation

Five hundred grams of shade dried powdered stem bark was repeatedly extracted with ethanol at room temperature. The combined extract (6 l) was concentrated under reduced pressure to get 40 g of brownish viscous material which was first partitioned between hexane/$H_2O$ and later EtOAc/$H_2O$. The concentrated EtOAc extract (15 g) was chromatographed over a silica gel column and eluted with hexane, hexane–benzene mixtures, benzene, benzene–chloroform mixtures, chloroform, chloroform–EtOAc mixtures, EtOAc and finally with EtOAc–MeOH mixtures to get compounds **1**–**10** and they were further purified by Prep.TLC. Compounds **1** (100 mg), **2** (8 mg), **3** (10 mg) and **4** (500 mg) were obtained from $C_6H_6$–$CHCl_3$ fractions, **5** (10 mg), **6** (10 mg), **7** (10 mg), from $CHCl_3$–EtOAc fractions, **9** (20 mg) and **10** (15 mg) from EtOAc–MeOH fractions. Compound **8** (15 mg) was deposited as white heavy crystals from earlier EtOAc–MeOH fractions.

### 3.4. Cardiobutanolide (*8*)

White crystals (from acetone). Mp 189–190 °C, $[\alpha]_D^{24}+6.4$ (c, 0.28, MeOH) $IR_{max}^{KBr}$ $cm^{-1}$: 3432, 1731, 1600. $^1H$ NMR ($d_6$-acetone) : Table 1. $^{13}C$ NMR($d_6$-acetone) Table 1. HR–FABMS: calc for $C_{13}H_{17}O_6$ $(M+H)^+$ 269.1025, found: 269.1073; FAB MS *m*/*z*: 269 $(M+H)^+$, 251, 239, 233, 215, 207, 197, 186, 167, 163, 149, 137, 133, 131, 115.

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