Remarkable Differences in the Reactivity of Echinadiol and Shiromodiol, Biologically Active Epimeric Germacrane Derivatives

Giovanni Appendino* and Paola Tettamanzi
Dipartimento di Scienza e Tecnologia del Farmaco, C. so Raffaello, 31; 10125 Torino, Italy
Pierluigi Gariboldi
Dipartimento di Scienze Chimiche, Via S. Agostino, 1; 62032 Camerino (MC), Italy

Treatment of echinadiol with a variety of acids gave a 8,9-secoguaiane aldol resulting from the cyclofragmentation of the ten-membered ring. Under these conditions its C-8 epimer (shiromodiol) did not react or underwent esterification of the hydroxy group at C-8. Allylic oxidation of both epimers occurred with oxidation of the allylic methyl to the formyl level and retention of configuration of the endocyclic *trans* (*Z*) double bond. However, whereas the enal from shiromodiol was stable, that from echinadiol smoothly underwent *Z–E* isomerization. Low-temperature NMR experiments showed that echinadiol and shiromodiol exist in solution as a mixture of parallel and crossed rotamers, whose ratio is *ca.* 1:5 for shiromodiol and 4:1 for echinadiol. Anchimeric stabilization of an incipient positive charge at C-10 or C-14 by the C-8 β-hydroxy group of echinadiol might be responsible for the observed differences in reactivity.

As part of a study on immunomodulators of plant origin, we investigated the conversion of the antifeedant germacrane derivative shiromodiol (**1**)[1,†] into its C-8 epimer echinadiol (**2**),[2] whose esters display immunostimulating activity.[2,3] Shiromodiol esters are available from several plants belonging to the *Umbelliferae* and *Lauraceae* families,[4] whereas echinadiol derivatives have so far been isolated only from *Parthenium integrifolium* L., an adulterant of *Echinacea purpurea* (L) Moench.,[5] a relatively expensive drug. Epimerization at C-8 was accomplished *via* an oxidation–reduction protocol, based on the chemoselective oxidation of the homoallylic hydroxy group of shiromodiol with activated MnO_2.[1] The subsequent reduction of the C-8 ketone gave a mixture of echinadiol and shiromodiol, whose ratio depended on the reducing agent and the solvent used for the reaction (see Experimental section). The best results were obtained with $NaBH_4$ in ethanol. Under these conditions, an easily separable‡ 2.4:1 mixture of compounds (**2**) and (**1**) was obtained. The overall yield of the two steps was 41% based on crude shiromodiol (see Scheme 1).

During the spectral characterization of echinadiol,§ we noticed that this compound was transformed into an aldehyde (rapid appearance of a non-exchangeable broad singlet at δ_H 9.84) by commercial samples of $CDCl_3$. A spectral characterization consistent with formula (**2**) could be obtained only in C_6D_6 or in $CDCl_3$ filtered over basic alumina immediately prior to the registration of the spectrum. The secoguaiane structure (**3**) was assigned to the rearranged product on the basis of a detailed analysis of its ^1H and ^{13}C NMR spectra (for the latter, see the Table). The stereochemistry at C-1 and C-5 was established by NOE difference experiments[6] on compound (**9**), the product of reduction and acetylation of the rearrangement product (**3**). A possible mechanism for the formation of product (**3**) from echinadiol (**2**) involves the transannular cyclization of the epoxyolefin moiety, followed (or accompanied) by fragmentation of the C-8–C-9 bond (Scheme 2). The skeletal rearrangement (**2**) ⟶ (**3**) is formally similar to that observed in the formation of caulolactones and two related spirolactones from C-8 keto germacrane precursors.[7,8] A 8,9-secoguaianolide is also known,[9] although its ultimate precursor has been suggested to be a guaiane derivative.[9] Shiromodiol and the acetate of echinadiol were stable in the samples of $CDCl_3$ that caused the cyclofragmentative rearrangement of echinadiol.

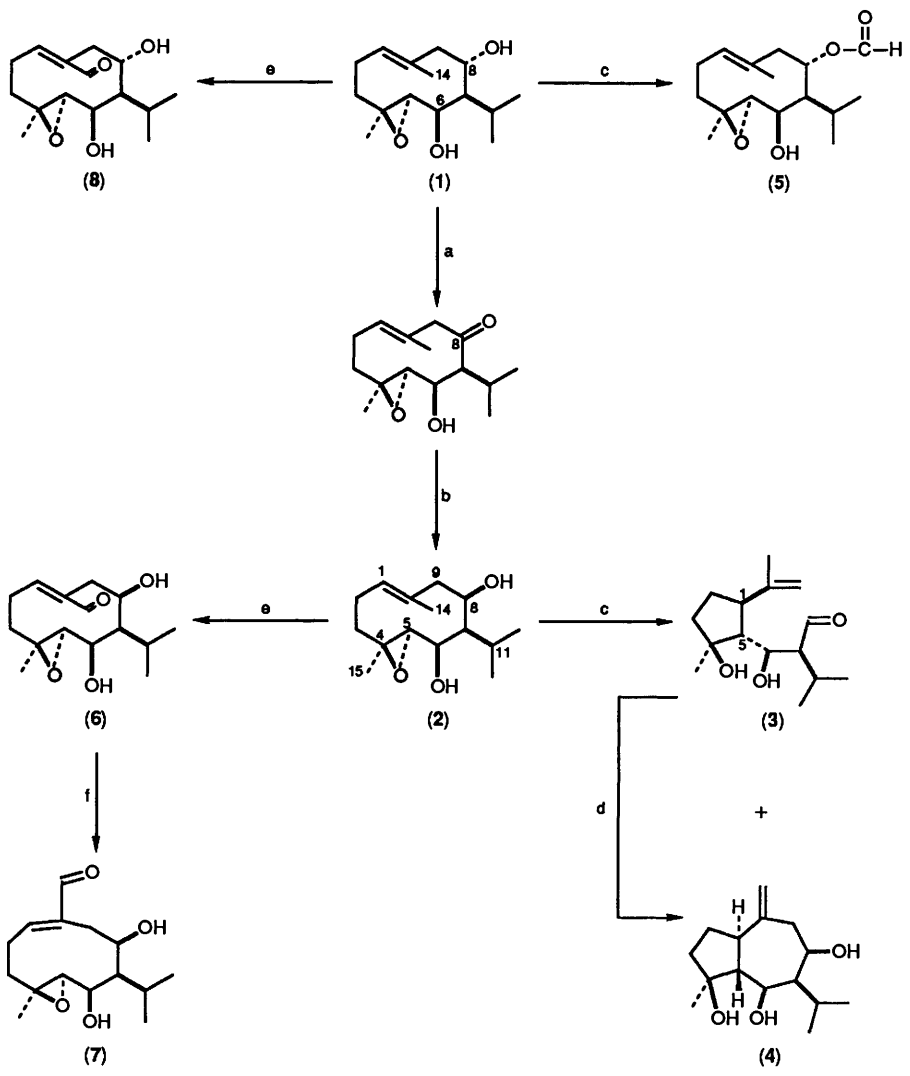
Attempts to reproduce this reaction under laboratory conditions using known concentrations of acids were frustrating, since most acidic reagents used (mineral acids, toluene-*p*-sulphonic acid (PTSA), BF_3·Et_2O) gave mixtures of bluish azulenoid products, with little, if any, rearrangement product (**3**) (TLC and NMR control). Good and reproducible yields were eventually obtained by using a dilute (1.2%) solution of formic acid in dichloromethane at room temperature. Besides product (**3**) (77%), minor amounts of the guaiane (**4**) were also isolated (14%) (Scheme 1). The *trans* relationship between 1-H and 5-H was evident from the value of their coupling constant (12.8 Hz).[10] Compound (**4**) most probably derives from a Prins-type intramolecular cyclization of compound (**3**) more than from the transannular cyclization of echinadiol (**2**), since compound (**3**) was unstable, and slowly rearranged to the guaiane (**4**) upon storage. After three months at refrigerator temperature, the conversion was *ca.* 25% (NMR control). We were unable to convert compound (**3**) into triol (**4**) in acceptable yields; treatment of the aldol (**3**) under acidic conditions stronger than those used for its formation from echinadiol (**2**) or simple prolongation of the latter reaction resulted in the development of a deep blue colour and the formation of a plethora of compounds which were not further investigated, but whose formation lowered the yield considerably. The formation of only one isomer at C-8 is connected to the conformationally locked arrangement of the aliphatic appendage at C-5 in the aldol (**3**), that leads to non-averaged values of $J_{5,6}$, $J_{6,7}$, and $J_{7,8}$ (11.5, *ca.* 0, and *ca.* 0 Hz, respectively). Owing to intramolecular hydrogen bonding with the hydroxy group at C-6, only one face of the carbonyl is in fact accessible.

The diacetates of echinadiol and shiromodiol were not cyclized by treatment with dilute solutions of formic acid; the

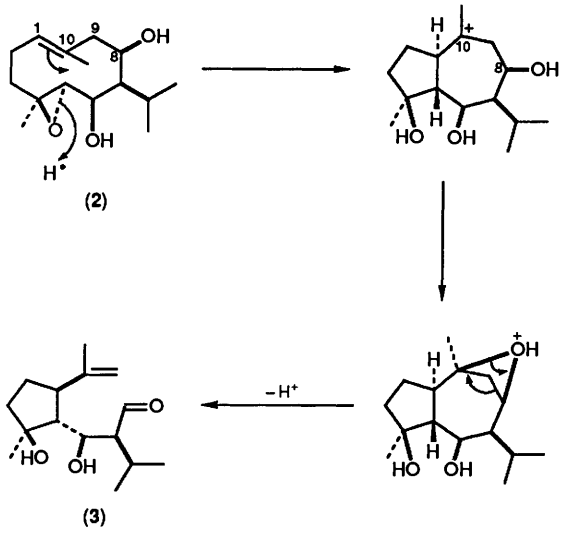
† Shiromodiol: (1*S*,2*R*,3*R*,4*S*,6*E*,10*S*)-6,10-dimethyl-3-(1-methylethyl)-11-oxabicyclo[8.1.0]undec-6-ene-2,4-diol.

‡ The large difference in R_f-value between compounds (**1**) and (**2**) (see Experimental section) is due to the presence, in echinadiol (**2**), of an intramolecular hydrogen bond between the hydroxy groups.[2]

§ Besides the preliminary results of an X-ray analysis,[2] no other physical or spectral data have been reported for compound (**2**).



Scheme 1. *Reagents and conditions:* (a) MnO_2, toluene; (b) $NaBH_4$, EtOH; (c) HCO_2H, CH_2Cl_2; (d) storage; (e) Bu^tOOH, SeO_2, CH_2Cl_2; (f) HCl.



Scheme 2. Possible mechanism for the acid-catalysed rearrangement of echinadiol (**2**) to apoechinadiol (**3**).

Figure 1. Conformational equilibrium of echinodiol (**2**); (**A**) parallel conformation; (**B**) crossed conformation.

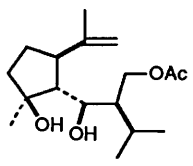
former was recovered unchanged, whereas the latter gave, besides unchanged starting material, a crystalline 8-*O*-formyl derivative (**5**) (Scheme 1). This compound, left in $CDCl_3$, quantitatively rearranged to the guaiane (**10**).

In order to rationalize the remarkable difference in reactivity between shiromodiol and echinadiol, their conformation in solution was investigated by NMR spectroscopy. Both compounds showed broad and unresolved spectra at room temperature, but heating at 60 °C sharpened most lines, and allowed assignment of the signals. Upon cooling to −40 °C, two uneven [*ca.* 5:1 for (**1**) and 4:1 for (**2**)] sets of signals appeared in the spectrum of each compound, corresponding to a parallel and a crossed geometry of the ten-membered ring (Figure 1).[4] These rotamers could be distinguished on the basis of a ROESY spectrum.[11] Especially diagnostic was the presence of correlation peaks between 5-H and the allylic methyl in the most abundant rotamer of echinadiol, and between 1-H and 5-H in the most abundant rotamer of shiromodiol. Thus, the

Table. ^{13}C NMR data (75.2 MHz; $SiMe_4$ as reference) assignments with the same sign in the same column are interchangeable; assignments are based upon multiplicity and chemical shift considerations as well as from comparison with literature data.[4]

Compound	(2)[a] major/minor	(3)[b]	(5)[b]	(6)[c]	(7)[d]
C-1	123.98/127.22 d	47.89 d*	A	150.15 d	153.56 d
C-2	22.36/24.73 t	26.95 t	24.29 t	23.00 t	26.10 t
C-3	35.88/38.32 t	40.84 t	38.45 t	38.82 t	35.69 t+
C-4	62.47/62.47 s	81.37 s	59.55 s	58.90 s	59.10 s
C-5	68.35/69.93 d	52.36 d*	68.63 d	68.92 d	64.87 d
C-6	72.61/72.79 d*	74.12 d	72.23 d*	73.18 d*	71.38 d*
C-7	47.83/48.54 d	59.43 d	49.57 d	49.81 d	46.67 d
C-8	76.97/75.88 d*	207.16 d	71.44 d*	75.00 d*	75.10 d*
C-9	46.47/42.30 t	112.07 t	41.24 t	35.07 t	34.85 t+
C-10	137.72/132.14 s	148.83 s	129.39 s	138.88 s	141.60 s
C-11	31.67/30.73 d	27.76 d	26.23 d	31.58 d	28.62 d
C-12	22.16/22.50 q†	20.80 q†	23.76 q†	22.78 q†	21.74 q†
C-13	20.99/21.23 q†	21.06 q†	21.51 q†	21.04 q†	21.50 q†
C-14	16.24/16.44 q	18.22 q	16.50 q	190.52 d	194.99 d
C-15	20.99/21.23 q	21.83 q	20.38 q	16.28 q	21.50 q
Formate			161.09 d		

[a] $CDCl_3$, −40 °C. [b] C_6D_6, room temperature. [c] C_6D_6, +60 °C. [d] $CDCl_3$, room temperature. A: signal not detected.



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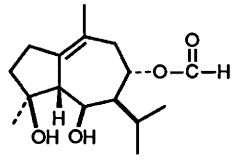

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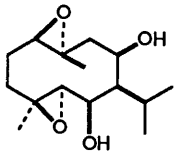

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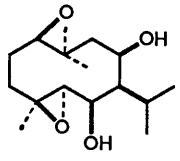

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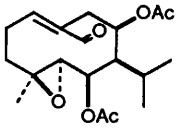

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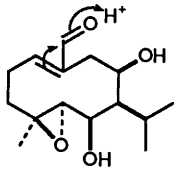

(14)

major conformation in solution is the parallel one for echinadiol, and the crossed one for shiromodiol. These geometries are also those adopted by echinadiol[2] and the mono- and di-esters of shiromodiol[1b,12] in the solid state.

Notwithstanding the presence of a dynamic conformational process, the secoguaiane (**3**) obtained from the acidic treatment of echinadiol (**2**) was stereoisomerically uniform. The *trans* relationship between 1-H and 5-H showed that compound (**3**) derives from the parallel conformation of echinadiol (**2**), which has these protons *anti*. The formally related rearrangement of some C-8 keto germacranes to spirolactones gave, instead, epimers at C-1,[7,8] showing that in this case the parallel and the crossed conformation are both reactive. Products derived from both conformations of the ten-membered ring were also obtained on treatment of echinadiol with peracids [(**11**) from the parallel conformation, (**12**) from the crossed].

In spite of the presence of a parallel rotamer in solution, no rearrangement of shiromodiol to compound (**3**) took place. This suggests that the difference in reactivity between compounds (**1**) and (**2**) cannot be explained exclusively on the basis of a different ratio between crossed and parallel conformations, pointing instead to a more direct role of the hydroxy group at C-8 in the course of the reaction. The reactivity of only one rotamer of echinadiol, and the stability of the epoxyolefinic system of shiromodiol and the acetate of echinadiol under the conditions causing rearrangement of echinadiol to compound (**3**), might in fact be the consequence of anchimeric assistance of the C-8 hydroxy group in the transannular opening of the oxirane ring. This generates a positive charge at C-10 (Scheme 2), which in the parallel conformation of echinadiol can be stabilized by the homoallylic synperiplanar hydroxy group at C-8 through the formation of a protonated oxetane (Scheme 2 and Figure 2). Intermediates of this type are documented,[13] and have been postulated to explain the stereochemical course of certain electrophilic additions to double bonds.[14] In the crossed conformation of echinadiol, C-10 and the hydroxy group at C-8 are synclinal and more distant (Figure 2), whereas in shiromodiol no interaction is possible, since C-10 and the hydroxy group at C-8 are *anti*. The stabilization of a nearby positive charge by the C-8 hydroxy group might also be responsible for the different stability of the products of allylic oxidation of compounds (**1**) and (**2**). The reaction occurred with oxidation of the allylic methyl to the formyl level and retention of configuration of the *trans* (*Z*) configuration of the endocyclic double bond in both epimers. However, whereas the enals from shiromodiol and the diacetate of echinadiol [(**8**) and (**13**), respectively] were stable, that from echinadiol, compound (**6**), smoothly underwent *trans*(*Z*)–*cis*(*E*) isomerization in solution. The reaction was accelerated by the addition of small amounts of mineral acids, and presumably proceeds *via* protonation of the aldehydic carbonyl, followed by double-bond shift (**14**) and reprotonation, eventually giving the thermodynamically more stable[15] *cis* (*E*)-isomer (**7**). Isomers (**6**) and (**7**) displayed quite different conformational features: the *trans* isomer (**6**) was flexible and showed broad and unresolved NMR signals at room temperature, whereas the *cis* isomer (**7**) was rigid and showed a spectrum consisting of sharp lines, unaffected by changes of temperature within the range −40 °C to +60 °C.

Figure 2. Newman projection along the C(8)–C(9) bond in the parallel (**A**) and crossed (**B**) conformations of echinadiol (**2**).

Inspection of the ROESY spectrum showed that isomer (**7**) adopts a conformation with the formyl and the methyl at C-4 *anti*. In the parallel conformation of the flexible *trans* aldehyde (**6**), the hydroxy group at C-8 can assist the protonation of the carbonyl, anchimerically stabilizing the positive charge at C-14 [see Figure 1, (**A**)], and thus making the reaction easier.

The results presented here show how intramolecular interactions can dramatically alter the reactivity of relatively small but heavily substituted molecules, potentially also affecting their biological properties.

Experimental *

M.p.s were determined on a Büchi SMP 20 apparatus and are uncorrected; optical rotations were measured on a Perkin-Elmer 141 automatic polarimeter. UV spectra were taken on a Beckman DB-GT spectrophotometer. IR spectra were recorded on a Perkin-Elmer model 237 spectrophotometer. Electron-impact (EI) mass spectra were taken on a Varian Mat CH7A apparatus; chemical ionization (CI) mass spectra were taken on a VG EQ 70/70 apparatus. ^1H and ^{13}C NMR spectra were obtained on a Varian VXR 300 spectrometer (300 and 75.4 MHz respectively) with $SiMe_4$ as reference [data for compounds (**2**), (**3**), and (**5**)–(**7**) are given in the Table]. The ROESY spectra were accumulated and processed in the phase-sensitive mode. 1 K data memory was used in the F2 dimension, and 256 T_1 increments, zero-filled to 1 024, before Fourier transformation. The mixing time was 0.8 s. Silica gel 60 (70–230 mesh, Merck) was used for column chromatography; a Waters microporasil column (80 × 3 cm) was used for preparative HPLC, with detection by a Waters differential refractometer 3401. All solvents used for chromatography were bought as technical grade, and distilled before use. Dry CH_2Cl_2 was distilled from CaH_2; commercial $CDCl_3$ used for the registration of the reported spectra was filtered over basic alumina (Pasteur pipette). A mixture of shiromodiol esters was obtained from the fruits of *Laserpitium halleri* Krantz as reported in ref. 4. Shiromodiol (**1**) was obtained by saponification of this mixture with 5% methanolic potassium hydroxide.[4]

Epimerization of Shiromodiol (**1**) *to Echinadiol* (**2**).—To a solution of shiromodiol (**1**) (1.024 g) in toluene (50 ml) was added activated MnO_2 (8 g; Merck). The suspension was stirred at room temperature for 8 h, filtered over Celite, and evaporated, to give crystalline 8-dehydroshiromodiol (920 mg, 91%),[4] which was used for the next step without any further purification.

To a solution of crude 8-dehydroshiromodiol (2.019 g) in ethanol (50 ml) was added an excess of $NaBH_4$ (1.103 g). The course of the reaction was followed by TLC [chloroform–acetone (6:1) as developer; R_f 8-dehydroshiromodiol 0.54; R_f (**2**) 0.32; R_f (**1**) 0.09]. After being stirred for 4 h at room temperature, the reaction mixture was cooled (ice-bath) and diluted with water (150 ml). Saturated aq. NH_4Cl was then slowly added, and the suspension was extracted with CH_2Cl_2. After being washed with brine, the organic phase was evaporated and the semi solid residue was charged on a silica gel (25 g) column; elution with hexane–EtOAc (7:3) gave echinadiol (**2**) (916 mg, 45%), and elution with hexane–EtOAc (3:7) afforded shiromodiol (390 mg, 19%).

The reaction carried out at −78 °C gave a similar ratio between compounds (**1**) and (**2**), but (of course) was considerably slower. The reaction could not be carried out in methanol, since the reduction was slow compared with the decomposition rate of $NaBH_4$. In propan-2-ol with $NaBH_4$ and in ethanol with $Ca(BH_4)_2$ and KBH_4 the major product of the reduction was shiromodiol (TLC control).

Echinadiol (**2**) was obtained as crystals from EtOAc–hexane, m.p. 100 °C; $[\alpha]_D^{25}$ +6.9° (*c* 0.8, CH_2Cl_2); ν_{max}(KBr disc) 3 350, 3 040, 1 390, 1 270, 1 220, 1 100, 1 070, 1 050, 940, and 835 cm^{-1}; *m/z* (CI, isobutane) (rel. int.) 255 ($C_{15}H_{26}O_3$ + H)$^+$ (M + H)$^+$ (15%), 237 (30), 219 (100), 191 (25), 123 (70), 115 (60), 107 (45), and 95 (45); δ_H(C_6D_6; 60 °C; averaged spectrum) 5.28 (br m, 1-H), 4.14 (br s, 8-H), 3.67 (br d, *J* 7.0 Hz, 6-H), 3.28 (d, *J* 7.0, 5-H), 3.08 (br s, OH), 2.41 (dd, *J* 12.0 and 3.0 Hz, 9a-H), 1.70 (s, 14-H_3), 1.05 (s, 15-H_3), 0.92 (d, *J* 6.8 Hz, 12-H_3), and 0.87 (d, *J* 6.8, 13-H_3); δ_H($CDCl_3$; −40 °C) diagnostic resonances of the major rotamer: 5.30 (br m, 1-H), 4.32 (br s, 8-H), 3.69 (br m, 6-H), 3.19 (d, *J* 7.0 Hz, 5-H), 1.75 (br s, 14-H_3), and 1.23 (s, 15-H_3); of the minor rotamer: 5.55 (br d, *J* 8.2 Hz, 1-H), 4.10 (br s, 8-H), 3.51 (d, *J* 7.0 Hz, 5-H), 1.70 (br s, 14-H_3), and 1.15 (s, 15-H_3). The ratio between the two rotamers was estimated from the integration of the signal of 5-H.

Acid-catalysed Rearrangement of Echinadiol (**2**).—(a) *In $CDCl_3$*. Echinadiol (**2**) was dissolved in commercial samples of $CDCl_3$ (Merck; Carlo-Erba; Aldrich: stabilized with silver threads; Janssen without stabilizer). After 1 h at room temp. the signal of the aldehyde was already detectable (δ 9.84, br s) at 60 MHz. The reaction was complete (TLC control) after 6–24 h, depending on the sample of $CDCl_3$. At this point the solution had turned slightly bluish. The guaiane (**4**) was not present in the reaction mixture (NMR and TLC control).

(b) *In formic acid–CH_2Cl_2*. A sample of echinadiol (**2**) (100 mg) was dissolved in a 1.2% (by weight) solution of formic acid (98%) in CH_2Cl_2 (4 ml). After 48 h at room temperature, the mixture was diluted with CH_2Cl_2, and washed successively with saturated aq. $NaHCO_3$ and brine. Evaporation of the solvent left an oil, which was purified by column chromatography [silica gel (5 g); hexane–EtOAc (6:4)] to give compound (**3**) (77 mg, 77%) and compound (**4**) (14 mg, 14%).

(2*S*,3*S*)-3-Hydroxy-3-[(1*R*,2*S*,5*R*)-2-hydroxy-2-methyl-5-(1-methylethenyl)cyclopentyl]-2-isopropylpropanal (apoechinadiol) (**3**) was obtained as an oil, $[\alpha]_D^{25}$ −61° (*c* 1.9, CH_2Cl_2); ν_{max}(liquid film) 3 380, 3 070, 1 720, 1 640, 1 390, 1 305, 1 100, 1 070, 930, 890, and 835 cm^{-1}; *m/z* (CI isobutane) (rel. int.) 255 ($C_{15}H_{26}O_3$ + H)$^+$ (*M* + H)$^+$ (20%), 237 (50), 219 (95), 191 (60), 151 (40), and 123 (100); δ_H($CDCl_3$) 9.84 (br s, 8-H), 4.73 (m, 9-H_2), 3.93 (d, *J* 11.5 Hz, 6-H), 1.72 (s, 14-H_3), 0.97 (d, *J* 7.3 Hz, 12-H_3), and 0.86 (d, *J* 7.3 Hz, 13-H_3).

(1*S*,3a*R*,6*R*,7*R*,8*R*,8a*R*)-decahydro-7-isopropyl-1-methyl-4-methyleneazulene-1,6,8-triol (β-cycloechinadiol) (**4**) was also obtained as an oil, $[\alpha]_D^{25}$ −12° (*c* 1.2 CH_2Cl_2); ν_{max}(liquid film) 3 600, 3 080, 1 660, 1 410, 1 330, and 920 cm^{-1}; *m/z* (CI, isobutane) (rel. int.) 255 ($C_{15}H_{26}O_3$ + H)$^+$ (*M* + H)$^+$ (38%), 219 (80), 191 (70), and 123 (100); δ_H($CDCl_3$) 4.77 (br s 14-H_2), 4.30 (br t, *J* 3.9 and 2.8 Hz, 8-H), 4.14 (br s, 6-H), 1.27 (s, 15-H_3), 1.07 (d, *J* 7.2 Hz, 12-H_3), and 1.00 (d, *J* 7.2 Hz, 13-H_3).

Reduction and Acetylation of Apoechinadiol (**3**).—A sample of compound (**3**) (39 mg) was dissolved in methanol (3 ml) and $NaBH_4$ (a few mg) was added. After the mixture had been

* Throughout, locants for protons in the NMR spectra refer to that carbon numbering scheme shown for compounds (**1**) and (**2**).

stirred at room temperature for a few min, saturated aq. NH_4Cl was added, and the mixture was extracted with CH_2Cl_2. After evaporation of the solvent, the residue was filtered through a short pad of silica gel, to afford a triol (39 mg, 99%), which was treated with pyridine (0.5 ml) and Ac_2O (0.5 ml). After 6 h the reaction mixture was worked up by the addition of ice and a few drops of methanol, and extracted with CH_2Cl_2. The residue obtained by evaporation of the solvent was chromatographed on silica gel (5 g). Elution with hexane–EtOAc (7:3) gave compound (**9**) (28 mg, 62%) as an oil, $[\alpha]_D$ +13° (*c* 0.83, CH_2Cl_2), ν_{max}(liquid film) 3 420, 3 070, 1 740, 1 645, 1 470, 1 375, 1 310, 1 250, 1 140, 1 035, 980, and 890 cm^{-1}; *m/z* (CI, isobutane) (rel. int.) 299 $(C_{17}H_{30}O_4 + H)^+$ $(M + H)^+$ (25%), 263 (28), 203 (65), 159 (100), 123 (65), and 99 (95); $\delta_H(CDCl_3)$ 4.68 and 4.64 (br s, 9-H^a and 9-H^b),4.42 (dd, *J* 12.0 and 3.2 Hz, 8-H^a), 4.07 (dd, *J* 12.0 and 3.2 Hz, 8-H^b), 3.95 (dd, *J* 10.1 and 1.3 Hz, 6-H), 2.22 (m, 1-H), 2.10 (t, *J* 10.1 Hz, 5-H), 2.05 (s, OAc), 1.73 (s, 14-H_3), 1.34 (s, 15-H_3), 0.98 (d, *J* 7.3 Hz, 12-H_3), and 0.94 (d, *J* 7.3 Hz, 13-H_3). NOE difference spectra were obtained by irradiation of the signals of 9-H^a and -H^b. Enhancements were observed for the signals of 1-H, 5-H, and the allylic methyl.

Acetylation of β-*Cycloechinadiol* (**4**).—A sample of compound (**4**) (14 mg) was treated with pyridine (0.5 ml) and Ac_2O (0.5 ml). After 48 h the reaction mixture was worked up by the addition of ice and a few drops of methanol. Extraction with CH_2Cl_2 gave an oil, which was purified by column chromatography [silica gel (2.5 g); eluant hexane–EtOAc (8:2)]; the diacetate of compound (**4**) was obtained as an oil (7 mg, 38%). This compound showed an NMR spectrum better resolved in the aliphatic region than that of the parent (**4**): $\delta_H(CDCl_3)$ 5.40 (dd, *J* 7.2 and 3.2 Hz, 6-H), 5.17 (td, *J* 9.2 and 2.4 Hz, 8-H), 4.92 (br s, 14-H^a), 4.86 (br s, 14-H^b), 2.60 (m, 9-H_2), 2.16 (dd, *J* 12.8 and 7.2 Hz, 5-H), 2.06 and 2.04 (each s, OAc), 1.51 (dt, *J* 10.0 and 3.6 Hz, 7-H), 1.31 (s, 15-H_3), 0.95 (d, *J* 7.0 Hz, 12-H_3), and 0.91 (d, *J* 7.0 Hz, 13-H_3).

Treatment of Apoechinadiol (**3**) *with Acids*.—A sample of compound (**3**) (40 mg) was dissolved in a 10^{-4}M solution of PTSA in CH_2Cl_2 (1 ml). After 50 min, TLC analysis showed the complete disappearance of the starting material, and the reaction mixture was worked up by the addition of solid $NaHCO_3$. After filtration, and evaporation of the solvent, the residue was chromatographed on silica gel (3 g). Elution with hexane–EtOAc (7:3) gave compound (**4**) (5.2 mg, 12%), identified by NMR (270 MHz) spectroscopy and TLC with an authentic sample. Several other products were present in the reaction mixture. The TLC profile [$CHCl_3$–acetone (6:1)] of the mixture of products obtained on treatment of echinadiol (**2**) under the same conditions was identical with that obtained from apoechinadiol (**3**).

Treatment of Shiromodiol (**1**) *with Formic Acid in* CH_2Cl_2.—A sample of shiromodiol (**1**) (100 mg) was dissolved in a 1.2% solution of formic acid in CH_2Cl_2 (4 ml). After 48 h, the reaction mixture was worked up as described for compound (**2**). The oil obtained was purified by column chromatography [silica gel (5 g)] to give the formate (**5**) (72 mg, 65%) and starting material (17 mg recovery). ^1H NMR analysis of the crude reaction mixture (C_6D_6; 270 MHz) did not show the presence of aldehydic signals.

8-*O*-Formylshiromodiol (**5**) was obtained as crystals m.p. 118 °C (from hexane); $[\alpha]_D^{25}$ −34° (*c* 1.5, CH_2Cl_2); ν_{max}(KBr disc) 3 430, 1 705, 1 380, 1 200, 1 195, 970, and 910 cm^{-1}; *m/z* (CI, isobutane) (rel. int.) 265 $(C_{16}H_{26}O_4 - H_2O + H)^+$ (5%), 237 (40), 219 (100), 201 (60), 191 (45), 81 (70), and 69 (65); $\delta_H(C_6D_6$; 60 °C) 7.70 (s, formate), 5.38 (dd, *J* 12.0 and 5.4, 8-H), 4.91 (br t, *J* 7.5 Hz, 1-H), 3.62 (m, 6-H), 2.77 (d, *J* 7.2 Hz, 5-H), 1.91 (br s, 14-H_3), 1.08 (d, *J* 6.8 Hz, 12-H_3), 0.94 (s, 15-H_3), and 0.91 (d, *J* 6.8 Hz, 13-H_3).

The germacrane (**5**), left for *ca.* 48 h in $CDCl_3$, quantitatively rearranged to the guaiane (**10**), $\delta_H(CDCl_3)$ 8.12 (s, formate), 5.31 (br m, 8-H), 3.57 (dd, *J* 7.0 and 2.3 Hz, 6-H), 1.78 (br s, 14-H_3), 1.13 (s, 15-H_3), 1.10 (d, *J* 6.4 Hz, 12-H_3), and 0.95 *J* 6.4 Hz, 13-H_3).

Epoxidation of Echinadiol (**2**).—To a solution of echinadiol (**2**) (50 mg) in dry CH_2Cl_2 (3 ml) were added anhydrous sodium acetate (8.1 mg, 0.5 mol equiv.) and *m*-chloroperbenzoic acid (85%; 56.5 mg, 1.5 mol equiv.). The reaction mixture was stirred 1 h at room temperature under nitrogen, and then worked up with 5% aq. Na_2CO_3 and brine washes. After removal of the solvent, the residue was crystallized from hexane–diethyl to give compound (**11**) (27 mg). The mother liquors were chromatographed [silica gel (2 g); eluant hexane–ErOAc (6:4)] to give a further crop (4 mg) of compound (**11**) and its epimer (**12**) (2 mg). The overall yield was 58% for compound (**11**) and 4% for compound (**12**). The major epoxide (**11**) was a crystalline solid, m.p. 147 °C; $[\alpha]_D^{25}$ −56° (*c* 1.5, $CHCl_2$); ν_{max}(KBr disc) 3 270, 1 470, 1 455, 1 395, 1 105, 1 050, 935, 840, and 775 cm^{-1}; $\delta_H(CDCl_3)$ 4.32 (dt, *J* 6.0 and 1.1 Hz, 8-H), 3.72 (br d, *J* 7.6 Hz, 6-H), 3.66 (br s, OH), 3.57 (d, *J* 7.6 Hz, 5-H), 3.28 (br s, OH), 3.05 (dd, *J* 10.0 and 7.8 Hz, 1-H), 2.62 (dd, *J* 12.4 and 6.0 Hz, 9-H^a), 2.31 (m, 2-H^a), 2.14 (dd, *J* 8.0 and 6.2 Hz, 2-H^b), 1.83 (m, 11-H), 1.50 and 1.39 (each s, 14- and 15-H_3), 1.07 (!) (br d, *J* 12.4 Hz, 9-H^b), 0.96 (d, *J* 7.2 Hz, 12-H_3), and 0.92 (d, *J* 7.2 Hz, 13-H_3).

The minor epoxide (**12**) had the following NMR data: $\delta_H(CDCl_3)$ 4.13 (td, *J* 6.2 and 3.0 Hz, 8-H), 3.81 (br d, *J* 8.0 Hz, 6-H), 3.67 (d, *J* 8.0 Hz, 5-H), 3.58 (dd, *J* 10.2 and 0.8 Hz, 1-H), *ca.* 3.00 and 2.70 (each br s, 2 × OH), 2.28 (dd, *J* 12.0 and 6.2, 9-H^a), 1.42 and 1.28 (each s, 14- and 15-H_3), 1.03 (d, *J* 7.2 Hz, 12-H_3), and 0.99 (d, *J* 7.2 Hz, 13-H_3).

The stereochemistry of the epoxide ring was assessed with the aid of the ROESY spectrum, which showed for the major epoxide (**11**) a correlation peak between the signals of 5-H and 14-H_3, thus showing that this epimer (**11**) derives from the parallel conformation of echinadiol (**2**).

Allylic Oxidation of Echinadiol (**2**).—To a suspension of SeO_2 (22 mg, 0.195 mmol, 0.5 mol equiv. in dry CH_2Cl_2 (0.3 ml) was added t-butyl hydroperoxide (0.107 ml, 0.78 mmol, 2 mol equiv.). When all the SeO_2 had dissolved, a solution of echinadiol (100 mg, 0.39 mmol) in dry CH_2Cl_2 (3 ml) was added, and the reaction mixture was stirred at room temperature under nitrogen for 19 h. After dilution with CH_2Cl_2, the reaction mixture was washed with brine and evaporated. The residue was purified by column chromatography [silica gel (5 g); hexane–EtOAc (6:4)] to give compound (**6**) as a powder (40 mg, 38%). Crystallization from acetone–diethyl ether gave an analytical sample, m.p. 131 °C; $[\alpha]_D^{25}$ +64° (*c* 1.1, CH_2Cl_2); λ_{max}(EtOH) 238 nm; ν_{max}(KBr disc) 3 360, 3 025, 1 670, 1 630, 1 425, 1 370, 1 100, 1 072, 1 060, 975, 855, and 830 cm^{-1}; *m/z* (CI, isobutane) (rel. int.) 269 $(C_{15}H_{24}O_4 + H)^+$ $(M + H)^+$ (25%), 207 (30), 203 (30), 189 (50), 179 (40), and 153 (100); $\delta_H(C_6D_6$; 60 °C) 9.86 (br s, 14-H), 6.50 (br s, 1-H), 4.12 (br m, 8-H), 3.60 (d, *J* 7.1 Hz, 6-H), 3.25 (br s, 5-H), 0.96 (d, *J* 6.8 Hz, 12-H_3), 0.94 (d, *J* 6.8 Hz, 13-H_3), and 0.90 (s, 15-H_3). In $CDCl_3$ compound (**6**) was quantitatively isomerized in 3 days at room temperature into compound (**7**) (shift of the aldehydic proton from δ_H 10.12 to 9.42). Another compound (*ca.* 15%), having the aldehydic proton resonance at δ_H 9.31, was also formed. This compound could not be further characterized.

Allylic Oxidation of Shiromodiol (**1**) *and the Diacetate of Echinadiol*.—The same procedure employed for echinadiol (**2**) was applied. The yield was 41% for 14-oxoshiromodiol[1] (**8**) and 28% for 14-oxoechinadiol diacetate (**13**). In the case of echinadiol diacetate, the 14-hydroxy derivative was also

isolated (27%). Compound (**13**) is an oil, $\delta_H(CDCl_3$; room temperature) 10.20 (br s, 14-H), 6.96 (br s, 1-H), 5.46 (m, 8-H), 4.93 (dd, *J* 7.6 and 2.1 Hz, 6-H), 3.19 (br d, *J* 7.6 Hz, 5-H), 2.11 and 1.99 (each s, OAc), 1.18 (v br s, 15-H_3), 1.00 (d, *J* 6.7 Hz, 12-H_3), and 0.84 (d, *J* 6.7 Hz, 13-H_3). The solutions of compounds (**8**) and (**13**) used for the characterization of these compounds showed no sign of decomposition after 1 week.

Isomerization of 14-*Oxoechinadiol* (**6**).—A sample of compound (**6**) (56 mg) was dissolved in CH_2Cl_2 (5 ml) and conc. HCl (0.050 ml) was added. After 16 h at room temperature, the reaction mixture was worked up by the addition of solid $NaHCO_3$ and was then filtered. After evaporation of the solvent, the residue was purified by HPLC [hexane–EtOAc (5:5)] to give the isomer (**7**) (22 mg, 39%) and starting material (10 mg recovery), $\delta_H(CDCl_3)$ 9.42 (d, *J* 1.7 Hz, 14-H), 6.70 (dd, *J* 7.2 and 2.6 Hz, 1-H), 5.00 (br t, *J* 7.7 Hz, 8-H), 3.70 (br d, *J* 7.6 Hz, 6-H), 3.47 (d, *J* 7.6 Hz, 5-H), 1.15 (s, 15-H_3), 0.99 (d, *J* 6.7 Hz, 12-H_3), and 0.96 (d, *J* 6.7 Hz, 13-H_3). Variable-temperature experiments and the ROESY spectrum were taken on a C_6D_6 solution, containing a small amount of $(CD_3)_2SO$ to increase the solubility. The ROESY spectrum displayed diagnostic correlation peaks between 14-H and 1-H [*cis*(*E*) stereochemistry of the endocyclic double bond] and between 1-H and 5-H (*anti* relationship between the formyl and the methyl at C-4).

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