Genepolide, a Sesterpene γ-Lactone with a Novel Carbon Skeleton from Mountain Wormwood (*Artemisia umbelliformis*)[⊥]

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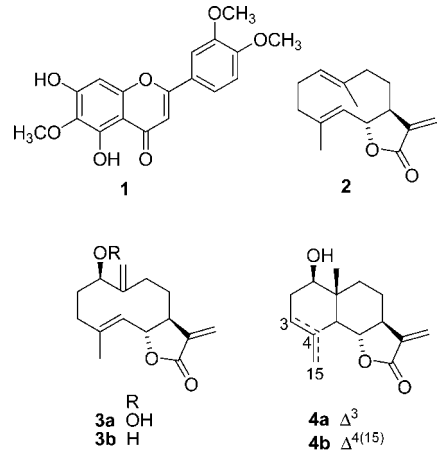
The sesterpene γ-lactone genepolide (**5**) has been isolated from a Swiss horticultural variety of mountain wormwood (*Artemisia umbelliformis*) developed as a thujones-free alternative to native Western Alps wormwoods for the production of liqueurs. Genepolide is the formal Diels−Alder adduct of the exomethylene-γ-lactone costunolide (**2**) and the diene myrcene (**6**), two poorly reactive partners in cycloaddition reactions, and its structure was elucidated through a combination of spectroscopic methods. An investigation on the thermal stability of mixtures of **2** and **6**, as well as considerations on the sensitivity of **2** to Brønsted and Lewis acids, suggests that **5** is a genuine natural product and that the Swiss chemotype of *A. umbelliformis* contains Diels−Alderase enzymatic activity that is lacking in native mountain wormwoods from Western Alps. Remarkable differences in thermal and acid-catalyzed reactions of the cyclodecadiene moiety of **2** and **5** suggest that quaternarization at C-11 has far-reaching effects on the reactivity of their homoconjugated medium-sized diene system. The wide occurrence of this structural motif in sesquiterpenoids makes this issue worth a systematic investigation.

The past two decades have witnessed an explosion of interest for secondary metabolites from food plants.[1] These studies were triggered by epidemiological correlations between semivegetarian diets and a reduced incidence of chronic and degenerative diseases, and have fostered a growing interest for healthy food by consumers and food companies.[2] Within this context, the Mediterranean diet has emerged as the archetypal healthy food style,[3] and enormous biomedical work has been done on olive oil and wine, the hallmarks of this dietary regime.[4] Surprisingly, little attention has been given to another important feature of the Mediterranean diet, namely, the consumption of wild, often bitter, herbs.[5] This trait has long been perceived of exclusive relevance for taste and flavor, but the recent discovery that taste receptors are expressed in the gastrointestinal tract[6] and are involved in the secretion of GLP-1 and in glucose homeostasis[7] suggests that taste-active compounds can have a biological profile that goes beyond chemoreception and involves glucose regulation, appetite control, and weight management.[7] Thus, bitter herbs have been traditionally used to stimulate appetite and treat cachexia,[8] while the beneficial effects of bitter wormwood extracts for the management of Crohn's disease suggests an even broader involvement in gastrointestinal health.[9]

Bitter vegetables and liqueurs prepared from bitter aromatic herbs are the major dietary sources of bitter compounds in the Western diet. Our studies in this area have focused on bitter terpenoids, characterizing a host of new sesquiterpenoids and diterpenoids from dietary plants,[10] and eventually identifying a human taste receptor (hTAS2R46) capable of binding this class of compounds.[11] As part of these studies, we have investigated a horticultural Swiss variety of mountain wormwood [*Artemisia umbelliformis* Lam. (Asteraceae)] characterized by the lack of thujones,[12] the major constituents of the essential oil of mountain wormwoods from Western Alps (*A. umbelliformis* and *A. genipy* Weber).[13] These plants are used for the production of genepy, the celebrated alpine bitter liqueur recently granted a Geographical Indication status by the European Community.[14] The wild chemotype of *A. umbelliformis* is characterized by the accumulation of bitter[11] sesquiterpene γ-lactones of the *cis* 8-olide type and of the polymethoxylated flavonoid eupatilin (**1**),[10c,d] a registered antiulcer drug in some Far East countries.[15] While retaining the presence of **1**, the Swiss chemotype of *A. umbelliformis* contained instead sesquiterpene γ-lactones of the *trans* 6-olide type. Thus, large amounts of the germacranolide costunolide (**2**) and minor amounts of its related oxygenated derivatives verlotorin (**3a**), artemorin (**3b**), reynosin (**4a**), and santamarin (**4b**) were isolated, a trait otherwise typical of *A. genipy*.[10a] Along with these compounds, relatively large amounts of a new lactone were present. This compound was absent in *A. genipy* and was characterized as the sesterpene γ-lactone **5**, named genepolide in consideration of the use of mountain wormwoods to produce the liqueur genepy.



Results and Discussion

Genepolide (**5**) was obtained as a colorless foam from fractions less polar than those containing costunolide (**2**). Its molecular

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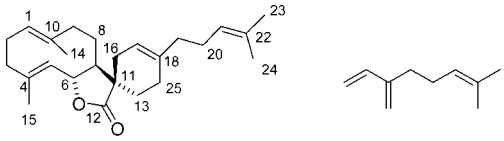
formula, $C_{25}H_{36}O_2$ (HREIMS), implied eight unsaturation degrees, while the IR spectrum retained the γ-lactone absorption band at 1766 cm^{-1}, typical of other bitter compounds from this plant.[10c,d] The ^1H NMR spectrum of **5** (Table 1) showed the presence of four relatively downfield shifted and broad methyl singlets (δ 1.38−1.67), a series of partially overlapped multiplets between δ 1.45 and 2.40, and five methine signals in the mid-field region of the spectrum (from δ 4.60 to 5.35). The ^{13}C NMR spectrum of **5** (Table 1) disclosed the presence of four carbon−carbon double bonds (eight signals between δ 117.3 and 139.5) and of one ester (lactone) carbonyl (δ 180.8), accounting for both oxygen atoms required by the molecular formula and for five of its eight degrees of unsaturation. Both the IR and ^{13}C NMR features of the carbonyl suggested a γ-lactone and not an ester moiety,[16] and therefore a C25 carbon connectivity was present. Taken together, these observations suggested that genepolide was a tricyclic sesterpene lactone, a very rare class of natural products.



5

6

A 2D NMR gradient-HSQC experiment made it possible to associate all the proton signals with those of the relevant carbon atoms. As a result, six carbon atoms resulted to be unprotonated (five sp^2 and a single sp^3 carbon, resonating at δ 44.5), while the remaining 19 carbons were all protonated. Among them, four mid-field methines were vinylic, while the fifth signal in this region was an oxymethine (δ_H 4.60, δ_C 79.3). The proton multiplets of the ^1H NMR spectrum of **5** could be arranged in five distinct spin systems (A−E, shown in bold in Figure 1) through the use of a 2D COSY spectrum. The $^{2,3}J_{C,H}$ gradient-HMBC experiment was instrumental in connecting these moieties and assembling the structure of **5**. In particular, the three gHMBC cross-peaks between both H_3-14 and H_3-15 methyls (Figure 1) established two links between fragments A and B, defining a dimethylcyclodecadiene subunit, while a γ-lactone ring was merged to this system on the basis of gHMBC cross-peaks between H-6 and H-7. The α-carbon of this lactone ring, the quaternary sp^3 carbon resonating at δ 44.5, should be the spiro carbon atom connecting the lactone ring with a cyclohexene ring, as suggested by gHMBC cross-peaks between H_2-13 and H_2-16. Finally, the whole series of cross-peaks between H-17, H_2-25, H_3-23, and H_3-24 located the prenyl moiety at C-18, thus completing the structure of genepolide (**5**).

The *E* configuration at the endocyclic $\Delta^{1(10)}$- and Δ^4-double bonds was deduced on the basis of the 2D ROESY cross-peaks H-1/H-9 and H-5/H-3, respectively, while α-orientation of H-7 was assumed, as typical for sesquiterpene lactones from asteraceous plants.[10a] The ROESY experiment was also helpful in defining the relative configuration of the three adjacent stereogenic carbons C-6, C-7, and C-11: Finally, the cross-peak H-6/H-8a suggested the *trans* junction of the lactone ring, while the cross-peaks H-7/H_2-13 and H-6/H_2-16 defined the relative configuration at C-11.

Genepolide (**5**) can be viewed as the result of a Diels−Alder cycloaddition between costunolide (**2**), the most abundant constituent of the plant, and the monoterpene myrcene (**6**). Since the existence of Diels−Alderase enzymes is still debated,[17] it was not clear if **5** could be considered a genuine natural product or rather an artifact from the isolation and/or fractionation steps. Myrcene was detected only in trace amounts in the essential oil from the Swiss variety of *A. umbelliformis*,[18] but this observation was not perceived as relevant since the thermal treatment required for obtaining the essential oil could have trapped myrcene as a Diels−Alder adduct with costunolide, the major constituent of the plant. We therefore investigated the thermal stability of a mixture

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR Data of Genepolide (**5**) in $CDCl_3$

pos.	δ_H, mult., *J* in Hz	δ_C, mult.	pos.	δ_H, mult., *J* in Hz	δ_C, mult.
1	4.80, bd, 10.7	126.8, d	13a	1.95[a]	30.5, t
2a	2.24[a]	26.0, t	13b	1.65[a]	
2b	2.13[a]		14	1.38, s	16.2, q
3a	2.25[a]	39.4, t	15	1.64, s	17.1, q
3b	1.99[a]		16a	2.20[a]	27.1, t
4		139.5, s	16b	2.14[a]	
5	4.61[a]	128.3, d	17	5.35, bs	117.3, d
6	4.60[a]	79.3, d	18		138.1, s
7	1.78, bt, 7.8	57.4, d	19	1.99[a]	37.6, t
8a	1.85, bdd, 15.1, 7.8	25.1, t	20	2.06[a]	26.2, t
8b	1.45, bdd, 15.1, 7.5		21	5.06, bt, 6.4	124.2, t
9a	2.32, dd, 13.1, 7.5	41.6, t	22		131.5, s
9b	1.97[a]		23	1.61, s	17.7, q
10		137.0, s	24	1.67, s	26.0, q
11		44.5, s	25a	2.40, dd, 15.1, 7.0	24.8, t
12		180.8, s	25b	1.94[a]	

[a] Overlapped with other signals.

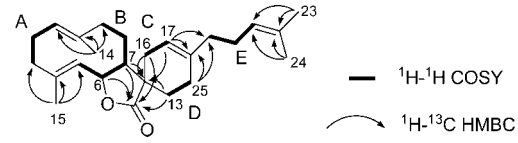
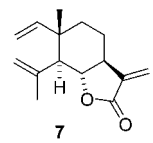


Figure 1. 2D NMR COSY and key $^{2,3}J_{H,C}$ HMBC cross-peaks of genepolide.

of costunolide and myrcene. Myrcene (**6**) has been reported to be a very poor diene,[19] while dienophilic behavior for sesquiterpene exomethylene-γ-lactones has been postulated to explain the occurrence of some bis-sesquiterpenoids.[20] No chromatographic or spectroscopic evidence of a reaction occurred when a toluene solution of myrcene and costunolide was kept in a sealed tube for 1 month, while prolonged (36 h) heating at 150 °C in *o*-dichlorobenzene resulted only in the formation of traces of the elemanolide derivative **7** (^1H NMR evidence), the result of a Cope rearrangement of the diallylic system of costunolide.[21] Diels−Alder reactions can be catalyzed by the addition of Lewis acids,[22] but, in our case, the addition of zinc chloride, a Lewis acid reported to increase the dienophilic behavior of myrcene,[19] to a cooled (0 °C) mixture of costunolide and myrcene led only to the formation of the cyclocostunolides **8a**−**c**,[23] without any trace of a Diels−Alder adduct, while heating in benzene gave only degradation products.



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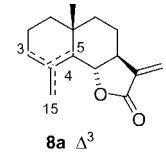
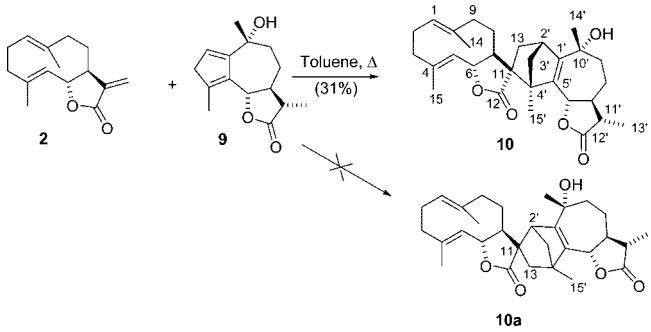


8a Δ^3
8b $\Delta^{4(15)}$
8c Δ^4

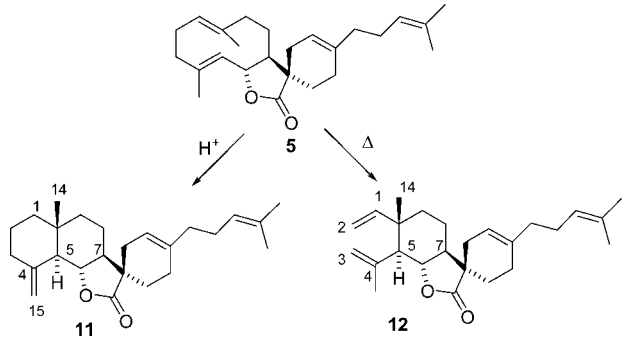
Faced with these observations and the lack of reactivity of mixtures of costunolide and myrcene, we eventually investigated if costunolide, when confronted with reactive dienes, could indeed behave as a dienophile.[20] Unsurprisingly, costunolide did not react with isoprene, but evidence of a reaction was observed with cyclopentadiene, and gentle heating of a mixture of costunolide and artabsin (**9**), a reactive cyclopentadienyl derivative,[24] cleanly afforded the Diels−Alder adduct **10** (Scheme 1) in a rewarding 31% conversion yield, showing that the exomethylene γ-lactone group can engage in cycloaddition reactions only with highly reactive partners. The structure of compound **10** was determined by inspection of MS and NMR data. In particular, the 2D NMR COSY, gHSQC, and gHMBC experiments were used to assign all ^1H and ^{13}C resonances (see Experimental Section) and to define the regiochemistry of the addition. In particular, COSY cross-peaks

Scheme 1. Formation of the Dimeric Lactone **10** by Thermal Cycloaddition of Costunolide (**2**) and Artabsin (**9**)[a]



[a] Regioisomer **10a** was not detected.

Scheme 2. Acid-Catalyzed Cyclization and Thermal Rearrangement of Genepolide (**5**)



of H-2′ with H_2-13 and the $^3J_{H,C}$ gHMBC cross-peak of H_3-15′ with C-11 excluded the alternative structure **10a** (Scheme 1). The 2D NMR ROESY spectrum of **10** was used to assess the relative configuration of the newly created stereogenic carbons, whose configuration shows that interactions occur in an *endo* fashion and according to the *ortho* rule. The triterpene dilactone **10** is structurally similar to arteminolides, a class of farnesyl-protein transferase inhibitors of relevance for cancer research.[25] Since artabsin is easily available in synthetically useful amounts from wormwood (*A. absinthium* L.)[26] and a vast array of exomethylene-γ-lactones can be isolated from commercially available medicinal and dietary plants,[27] the successful preparation of **10** points to the possibility of expanding the limited structure−activity relationships of arteminolides.[25]

During the study on the reactivity of costunolide in cycloaddition reactions, we became aware of a marked reactivity difference between the germacradienolide systems of costunolide (**2**) and genepolide (**5**). For example, while treatment of **2** with protic acids gave the mixture of eudesmane derivatives **8a**−**c**,[23,28] under similar conditions **5** cleanly afforded the single cyclization product **11** (Scheme 2). Interestingly, even more marked differences were observed in the thermal treatment of **2** and **5**. Thus, while heating of **2** at 200 °C in a sealed tube gave mainly a mixture of degradation products, under similar conditions **5** quantitatively afforded the elemanolide-type compound **12** (Scheme 2). In the realm of germacradienolides, this behavior has previously been reported for compounds having the C-15 methyl replaced by a formyl group[29] or with a *cis*-6-olide configuration.[30] It is not clear how quaternarization at C-11 could promote the Cope rearrangement of the germacradiene system, since the configuration of the elemanolide-type compound **12** (β-methyl at C-10 and α-hydrogen at C-5, as suggested by ROESY cross-peaks H_3-14/H-6 and H-5/H-7) shows the involvement of the same [$_1$D^{14},^{15}D$_6$] reactive conformation[31] of the cyclodecadiene ring as also observed in the transannular cyclization of both compounds.

In conclusion, the isolation of a formal Diels−Alder adduct between an unreactive diene such as myrcene (**6**) and an acid and thermally unstable poor dienophile such as costunolide (**2**) suggests that genepolide (**5**) is not an isolation artifact but a genuine natural product and that the biogenetic machinery of *A. umbelliformis* contains Diels−Alderase enzymatic activity. This observation is important for the hot debate on the existence of enzymes promoting cycloaddition reactions, since all previously reported Diels−Alder adducts involving exomethylene sesquiterpene-γ-lactones involve reactive dienophiles and not a poor partner such as myrcene.[17] In addition, remarkable differences were observed in the thermal and acid-catalyzed reactivity of the cyclodecadiene moiety of costunolide and genepolide, suggesting that quaternarization at C-11 has a dramatic effect on the reactivity of the medium-sized diene system of these compounds. Finally, the absence of genepolide in native wormwoods from Western Alps qualifies this compound and its degradation products **11** and **12** as critical markers for the detection of the alien Swiss chemotype in genepy from Western Alps.

Experimental Section

General Experimental Procedures. Low- and high-resolution EIMS spectra (70 eV) were performed on a VG Prospec (Fisons) mass spectrometer. ESIMS spectra were performed on a LCQ Finnigan MAT mass spectrometer. Optical rotations ($CHCl_3$) were measured at 589 nm on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (λ = 589 nm) and a 10 cm microcell. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were measured on a Varian INOVA spectrometer. Chemical shifts were referenced to the residual solvent signal ($CDCl_3$: δ_H 7.26, δ_C 77.0). Homonuclear ^{1}H connectivities were determined by the COSY experiment. One-bond heteronuclear ^{1}H−^{13}C connectivities were determined with the HSQC experiment. Two- and three-bond ^{1}H−^{13}C connectivities were determined by HMBC experiments optimized for a $^{2,3}J$ of 7 Hz. Through-space ^{1}H connectivities were evidenced using a ROESY experiment with a mixing time of 500 ms. Two- and three-bond ^{1}H−^{13}C connectivities were determined by gradient 2D HMBC experiments optimized for a $^{2,3}J$ of 9 Hz. Silica gel 60 (70−230 mesh) and Lichroprep RP-18 (25−40 mesh) were used for gravity CC. Reactions were monitored by TLC on Merck 60 F_{254} (0.25 mm) plates, which were visualized by UV inspection and/or staining with 5% H_2SO_4 in ethanol and heating. Organic phases were dried with Na_2SO_4 before evaporation.

Plant Material. The Swiss chemotype of *A. umbelliformis* was purchased from the Alpine farm La Freido (Stroppo, CN, Italy) of the Associazione Genepi Occitan. A voucher specimen (GA20082) is kept at the Novara Laboratories.

Extraction and Isolation of Genepolide (5). The powdered plant material (nonwoody aerial parts, leaves, and flowers, 631 g) was extracted with acetone (3 × 7.5 L) at room temperature, affording 57 g (9.0%) of a black syrup, part of which (6.0 g) was purified by filtration over RP-18 silica gel (30 g) with MeOH to remove fats and pigments. The methanol filtrate was evaporated, affording 3.52 g of a brownish paste. The latter was fractionated by gravity column chromatography (CC) on silica gel (90 g, petroleum ether−EtOAc gradient). Fractions eluted with petroleum ether−EtOAc (9:1) afforded 127 mg of crude **5**, further purifed by gravity CC on neutral alumina to obtain 80 mg of **5** (0.12%). Fractions eluted with petroleum ether−EtOAc (8:2) afforded, after crystallization from ether, 1.20 g of costunolide (**2**) (1.8%). Fractions eluted with petroleum ether−EtOAc (7:3) were crystallized from ether to afford 41 mg (0.06%) of eupatilin (**1**). The mother liquors and the fractions eluted with petroleum ether−EtOAc (6:4) were further purified by gravity CC on silica gel to afford 10 mg of crude verlotorin (**3a**), a ca. 1:1 mixture of santamarin (**4a**) and reynosin (**4b**) (60 mg, 0.09%), and artemorin (**3b**, 29 mg, 0.04%).

Genepolide (5): colorless foam; $[\alpha]^{25}_D$ −7 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 1766, 1438, 1278, 1208, 1139, 979 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 1; ESIMS (positive-ion) *m*/*z* 391 $[M + Na]^+$; HREIMS *m*/*z* 368.2723 $[M]^+$ (calcd for $C_{25}H_{36}O_2$, 368.2715).

Cycloaddition of Costunolide (2) and Artabsin (9). An equimolecular mixture of artabsin (**9**, 107 mg, 0.43 mmol) and costunolide (**2**, 100 mg, 0.43 mmol) in toluene (1 mL) was heated at 60 °C. The course of the reaction was followed by TLC (petroleum ether−EtOAc, 8:2), monitoring the disappearance of **2**. After 48 h, the reaction was

worked up by evaporation, and the residue was purified by gravity CC on silica gel (petroleum ether−EtOAc gradient, from 8:2 to 6:4) to afford 29 mg of recovered **2** and 34 mg (31% based on the conversion of costunolide) of **10** as a white powder: mp 120−122 °C; IR (KBr) ν_{max} 1767, 1455, 1229, 1172, 1000, 943 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.50 (1H, bd, J = 8.0 Hz, H-6′); 4.86 (1H, bd, J = 10.5 Hz, H-1); 4.63 (1H, d, J = 7.8 Hz, H-5); 4.47 (1H, t, J = 7.8 Hz, H-6); 3.03 (1H, bs, H-2′); 2.42 (1H, m, H-9a); 2.35 (1H, m, H-7); 2.32 (1H, m, H-3a); 2.26 (1H, m, H-11′); 2.25 (1H, m, H-2a); 2.23 (1H, m, H-3′a); 2.10 (1H, m, H-2b); 2.00 (1H, m, H-3b); 2.00 (1H, m, H-9b); 1.93 (1H, m, H-8a); 1.86 (1H, m, H-7′); 1.71 (1H, m, H-8′a); 1.67 (3H, s, H-15); 1.65 (1H, m, H-13a); 1.57 (1H, m, H-8′b); 1.51 (1H, m, H-8b); 1.46 (3H, s, H-15′); 1.45 (1H, m, H-3′b); 1.40 (3H, s, H-14); 1.36 (3H, s, H-14′); 1.32 (1H, m, H-13b); 1.20 (1H, d, J = 7.0 Hz, H-13′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 182.0 (s, C-12); 179.8 (s, C-12′); 150.5 (s, C-1′); 146.1 (s, C-5′); 140.1 (s, C-4); 137.5 (s, C-10); 128.3 (d, C-5); 128.1 (d, C-1); 88.9 (d, C-6′); 79.4 (d, C-6); 73.1 (s, C-10′); 62.3 (s, C-11); 57.0 (s, C-4′); 53.7 (t, C-3′); 51.4 (d, C-7); 51.4 (d, C-7′); 42.1 (d, C-11′); 41.3 (t, C-9); 40.6 (t, C-9′); 39.9 (t, C-3); 35.9 (t, C-13); 32.5 (2′); 27.2 (14′); 26.8 (t, C-8); 25.8 (t, C-2); 25.6 (t, C-8′); 20.4 (15′); 17.8 (q, C-15); 16.9 (q, C-14); 13.2 (q, C-13′); ESIMS (positive-ion) m/z 503 $[C_{30}H_{40}O_5 + Na]^+$.

Transannular Cyclization of Genepolide (5). A solution of **5** (50 mg, 0.13 mmol) in $CHCl_3$ (5 mL) was irradiated in a quartz photochemical reactor using a low-pressure mercury lamp. The course of the reaction was monitored by TLC (petroleum ether−EtOAc, 95:5; $R_{f(5)} = 0.50$; $R_{f(11)} = 0.60$). After 20 min, the solvent was evaporated and the residue, analyzed by ^{1}H NMR, indicated complete conversion to **11**. An analytical sample was obtained by gravity CC on silica gel (petroleum ether−EtOAc, 98.5:1.5): IR (KBr) ν_{max} 1770, 1505, 1288, 1270, 1198, 980 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.38 (1H, bt, J = 7.0, Hz, H-17); 5.05 (1H, bt, J = 7.0 Hz, H-21); 4.91 (1H, bs, H-15a); 4.75 (1H, bs, H-15b); 4.10 (1H, t, J = 8.2 Hz, H-6); 2.30 (1H, m, H-25a); 2.20 (1H, m, H-16a); 2.12 (1H, m, H-16b); 2.12 (1H, m, H-5); 2.08 (2H, m, H-20a,b); 2.00 (2H, m, H-19a,b); 1.99 (1H, m, H-25b); 1.98 (1H, m, H-3a); 1.95 (1H, m, H-13a); 1.82 (1H, m, H-7); 1.67 (3H, bs, H-23); 1.65 (1H, m, H-13b); 1.64 (1H, m, H-3b); 1.60 (3H, bs, H-24); 1.51 (1H, m, H-9a); 1.45 (1H, m, H-8a); 1.40 (1H, m, H-2a); 1.35 (1H, m, H-1a); 1.30 (1H, m, H-8b); 1.22 (1H, m, H-9b); 1.21 (1H, m, H-2b); 1.06 (1H, m, H-1b); 0.90 (3H, s, H-14); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 179.9 (s, C-12); 137.8 (s, C-18); 136.8 (s, C-4); 132.0 (s, C-22); 124.5 (d, C-21); 118.0 (d, C-17); 109.3 (t, C-15); 77.9 (d, C-6); 57.0 (d, C-7); 56.0 (d, C-5); 44.5 (s, C-11); 37.9 (t, C-19); 37.8 (t, C-1); 37.7 (t, C-9); 36.2 (t, C-3); 30.8 (t, C-13); 30.8 (t, C-25); 27.1 (t, C-16); 26.4 (q, C-23); 26.2 (s, C-10); 26.2 (t, C-20); 24.5 (t, C-8); 24.0 (t, C-2); 22.0 (q, C-14); 18.2 (q, C-24); ESIMS (positive ion) m/z 391 $[C_{25}H_{36}O_2 + Na]^+$.

Under similar conditions, the reaction with costunolide (**2**) required a longer time (90 min) and afforded a ca. 3:6:1 mixture (^{1}H NMR analysis) of α-, β-, and γ-cyclocostunolides (**8a**−**c**), which was fractionated by gravity CC on silica gel (petroleum ether−EtOAc, 98:2, to separate the α- and β-isomers, and petroleum ether−EtOAc, 9:1, to elute the minor γ-isomer).

Cope Rearrangement of Genepolide (5). A sample of **5** (30 mg, 0.08 mmol) was heated under vacuum at 220 °C (oil bath) for 3 min. ^{1}H NMR analysis indicated quantitative conversion to **12**. Evaporation of the solvent afforded an off-white, amorphous foam: IR (KBr) ν_{max} 1770, 1440, 1376, 1213, 1135, 1001 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.76 (1H, dd, J = 17.3, 10.8 Hz, H-1); 5.36 (1H, bt, J = 7.0, Hz, H-17); 5.05 (1H, bt, J = 7.0 Hz, H-21); 4.99 (1H, bs, H-3a); 4.95 (1H, bd, J = 10.8 Hz, H-2a); 4.91 (1H, bd, J = 17.3 Hz, H-2b); 4.66 (1H, bs, H-3b); 4.21 (1H, t, J = 11.2 Hz, H-6); 2.40 (1H, m, H-25a); 2.24 (1H, d, J = 11.2 Hz, H-5); 2.20 (1H, m, H-16a); 2.14 (1H, m, H-16b); 2.06 (2H, m, H-20a,b); 1.99 (2H, m, H-19a,b); 1.95 (1H, m, H-13a); 1.95 (1H, m, H-25b); 1.79 (1H, m, H-7); 1.76 (3H, s, H-15); 1.67 (1H, m, H-13b); 1.66 (3H, bs, H-23); 1.63 (1H, m, H-8a); 1.59 (3H, bs, H-24); 1.57 (1H, m, H-9a); 1.45 (1H, m, H-8b); 1.39 (1H, m, H-9b); 1.04 (3H, s, H-14); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 181.7 (s, C-12); 148.6 (d, C-1); 141.2 (s, C-4); 137.7 (s, C-18); 131.7 (s, C-22); 124.2 (d, C-21); 116.8 (t, C-2); 115.1 (t, C-3); 111.5 (d, C-17); 78.8 (d, C-6); 56.6 (d, C-5); 54.5 (d, C-7); 42.6 (s, C-10); 44.7 (s, C-11); 40.1 (t, C-9); 37.6 (t, C-19); 30.5 (t, C-13); 26.5 (t, C-16); 26.2 (q, C-23); 25.9 (t, C-20); 24.8 (t, C-25); 24.0 (q, C-15); 23.0 (t, C-8); 18.6 (q, C-14); 18.2 (q, C-24); ESIMS (positive-ion) m/z 391 $[C_{25}H_{36}O_2 + Na]^+$.

Under similar conditions, costunolide (**2**) yielded a resinous mass, partly insoluble in organic solvents, and probably resulting from a polymerization process. The soluble fraction, when analyzed by ^{1}H NMR, showed a complex mixture.

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