11,13-METHYLENATION OF SESQUITERPENE EXOMETHYLENE--γ-LACTONES: SYNTHESIS OF SESQUITERPENE CYCLOPROPYL--γ-LACTONES

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Dedicated to the memory of Professor František Šorm.

11,13-Methylene derivatives of sesquiterpene exomethylene-γ-lactones were prepared by reaction of diazomethane and photolysis of the resulting pyrazoline adduct(s). Variable amounts of exomethylene- and ethylidene-γ-lactones were also formed in the photolysis reaction. These compounds could be removed after reaction with diethylamine and chromatography on a bilayer (basic alumina/silica gel) column. Also 11,13-methylene derivatives of acid- and thermolabile germacranolides could be prepared in good yield with this procedure. Results obtained using alternative methods of methylenation are reported. A comparative study of the reactivity towards nucleophiles of exomethylene- and cyclopropyl sesquiterpene-γ-lactones provided little support for the involvement of compounds of the latter class in "in vivo" alkylation reactions.

In spite of the inherent strain of a three-membered ring, cyclopropane derivatives are not rare in nature[1], and cyclopropyl intermediates play an important role in several biological processes, such as the head-to-head condensation of isoprenoid residues[2] and the formation of the plant hormone ethylene[3]. The cyclopropane ring is also an important feature of several biologically active natural products (pyrethrins, hypoglycins)[1], and cyclopropane derivatives have proved useful in the study of enzyme rections and in drug design[4]. In biologically active cyclopropane derivatives, the three membered ring can provide a suitable molecular framework for the receptor--substrate interaction, or alternatively act as a reacting group, mostly of the electrophilic type[4]. Owing to their homo-alkene nature, cyclopropanes bearing carbonyl group(s) can in fact undergo homo-Michael reactions, as shown in vitro for monoactivated cyclopropanes when a suitable electrophilic group is present[5].

Our interest in the chemistry of cyclopropane derivatives was prompted by the observation that the plant-hormone activity of sesquiterpene exomethylene-γ-lactones is maintained or even increased by methylenation[6] of the double bond ($A \rightarrow B$,

Scheme 1). The alleged mechanism for the plant hormone activity of sesquiterpene exomethylene-γ-lactones is the same proposed for their cytotoxic activity, that is, alkylation of bionucleophiles via Michael reaction[6,7]. On account of this similarity,
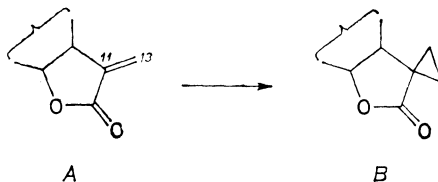
SCHEME 1   *A*   *B*

we hoped that substitution of a cyclopropane for the exomethylene might still give cytotoxic lactones. These, owing to a lesser reactivity towards nucleophiles[5], might act as more selective alkylating agents. We report here a high yield procedure for the 11,13-methylenation of sesquiterpene exomethylene-γ-lactones.

RESULTS AND DISCUSSION

Literature procedures for the methylenation of double bonds were tested on costunolide *I*, the most elementary germacranolide. Germacrane derivatives are acid- and thermolabile, and bear highly reactive endocyclic double bonds. Therefore a procedure applicable to compounds of this class is also expected to suit sesquiterpene lactones having inherently more stable carbocyclic skeletons. The 11,13-methylene derivative of costunolide *III* had already been prepared, albeit in low yield (15%), by pyrolysis[8,9] of its pyrazoline adduct *II*. Under these conditions the reaction is complicated by Cope rearrangement of the 1,5-diene system, and by cycloreversion of the pyrazoline adduct to the starting exomethylene-γ-lactone. Furthermore, ethylidene-γ-lactones, resulting from rearrangement of the diradical derived by extrusion of nitrogen, are also formed. As a result, a mixture of six compounds is obtained (*I, III — VII*). Spectroscopically pure *III* could be obtained from this mixture only with considerable difficulty. Even lower yields were obtained when the pyrolitic reaction was tested on more oxygenated germacranolides. Alternative procedures were thus investigated.

    Treatment of costunolide with 1·2 molar equivalents of Corey's reagent dimethyloxosulfonium methylide[10] resulted in an instantaneous reaction, with formation of the cyclopropane *III*. After working-up the reaction, *III* was the only compound present in the organic phase; however, the yield was low (20%), owing to the formation of water-soluble compounds that could not be characterized*. Attempts to

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*   Formation of acyl ylides has been reported as a side reaction in the cyclopropanation of α,β-unsaturated esters via sulfur ylides[11].

increase the yield varying the ratio between ylide and lactone, by slow addition of the reagents or by "inverse (ylide to lactone) addition" gave no results. Furthermore, in more functionalized substrates, the reaction was not chemoselective. Treatment of the bis-ketolactone umbellifolide[12] *VIII* with 1·2 equivalents of the ylide gave in fact, besides starting material, a 3 : 1 mixture of the cyclopropyl lactone *IX* and the epoxycyclopropane *X* (9 : 1 mixture of diastereomers at C-4).
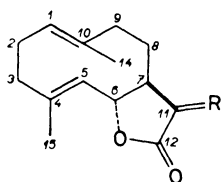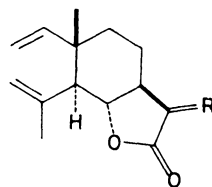

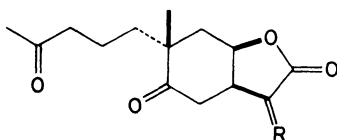*I*, R = =CH$_2$
III, R = —CH$_2$—CH$_2$—
IV, R = =CHCH$_3$ (*Z*)
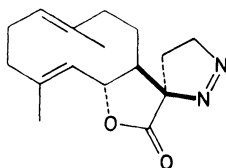
II

V, R = =CH$_2$
VI, R = —CH$_2$—CH$_2$—
VII, R = =CHCH$_3$ (*Z*)

VIII, R = =CH$_2$
IX, R = —CH$_2$—CH$_2$—

X

XI
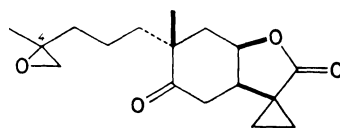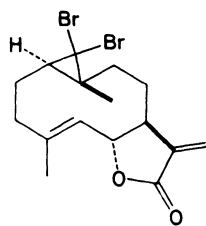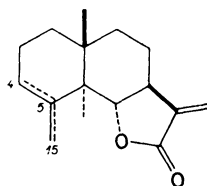
XII, $\Delta^{4,5}$
XIII, $\Delta^{5,15}$

As expected, treatment of costunolide with carbenoid species gave exclusively attack at the more electrophilic 1,10 double bond. Thus, treatment with Seyferth's reagent tribromomethyl phenyl mercury[13] gave the 1,10-dibromomethyl derivative *XI*, accompanied by a mixture of α- and β-cyclocostunolides *XII* and *XIII*, whose formation is presumably due to the Lewis acid nature of the reagent. The reaction

of costunolide with the carbene-metal complex derived from diazomethane and palladium(II) acetate[14] gave a complex mixture of products, containing both unreacted costunolide and its pyrazoline adduct. Good results were eventually obtained by photolysis[15] of the pyrazoline adduct *II*. Irradiation of *II* with a medium-pressure mercury lamp in toluene solution gave in fact a 75 : 10 : 15 mixture (¹H NMR (300 MHz) analysis) of the cyclopropyl lactone *III*, the ethylidene lactone *IV* and the exomethylene lactone *I*. In spite of a much simpler composition compared to that of the pyrolytic reaction, a thorough purification of the cyclopropyl lactone from the photolysis mixture was still difficult, and required HPLC. Costunolide and its cyclopropyl derivative, as well as most pairs of exomethylene and cyclopropyl lactones investigated, were in fact indistinguishable by TLC, and could be separated only by HPLC. Owing to the difficulty of this separation, the yield of NMR pure (300 MHz) *III* was only c. 40%. A much better purification procedure could be developped taking advantage of the high reactivity of exomethylene-γ-lactones towards nucleophiles. Thus, the crude mixture from the photolysis reaction was treated with an excess diethylamine in methanol at room temperature[16] for two hours, resulting in the quantitative conversion (NMR analysis) of the exomethylene lactone *I* into its Mannich base *XIV*. After removal of the solvent, the residue was

XIV   XV
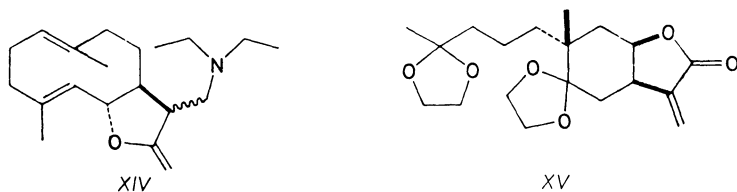
charged on a bi-layer column packed with basic alumina (top) to retain the silica gel unstable Mannich base, and with silica gel (bottom) to separate cyclopropyl- and ethylidene lactones*. Using this procedure, the yield of the cyclopropyl lactone *III* was almost 70% (based on the starting pyrazoline adduct). The ethylidene lactone *IV* required more drastic conditions to react with diethylamine (refluxing in methanol for 24 h), and formation of the Mannich base was not quantitative (NMR control). Under these conditions the cyclopropyl lactone *III* was unreactive**.

This protocol (reaction with diazomethane − photolysis of the resulting pyrazoline adduct(s) − reaction with diethylamine − column chromatography) was applied to the thirteen exomethylene-γ-lactones listed in Table I. The yield, based on the starting exomethylene lactones, ranged between 45 and 70%, depending on the

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*   Commercial (Merck) column chromatography silica gel was acidic enough to cause regeneration of exomethylene lactones from their Mannich bases.

**   Also esters of exomethylene carboxylic acids (e.g.: the methyl esters of costic and ilicic acids) were unreactive with diethylamine at room temperature.

ratio between cyclopropyl-, ethylidene- and exomethylene lactones in the photolysis step and on the difficulty of separating cyclopropyl- and ethylidene lactones by chromatography. As shown in Table I, this protocol could be succesfully applied to oxygenated germacranolides as well as to compounds bearing the most common functional groups encountered in naturally occurring sesquiterpene lactones. When a mixture of pyrazolines was obtained, the latter was used without any further purification. C(6)-*Trans* lactonized germacranolides gave only (*Z*)-ethylidene lactones (H-13 δ 6·10−6·40 ppm), whereas an almost equimolecular mixture of (*E*)- (H-13 δ 6·70−7·10 ppm) and (*Z*)-isomers was obtained in the other cases. The order of chromatographic elution was always (*Z*)-ethylidene lactone − cyclopropyl lactone − (*E*)-ethylidene lactone. The scale of the reaction was 100 mg − 1 g.

The procedure did not give good results with the secoeudesmanolide *VIII* and its bis-ketal derivative *XV*. For these compounds the ratio cyclopropyl-/(*E*)-isomer) ethylidene-/ and exomethylenelactone in the photolysis step was not só favourable (c. 5 : 3 : 2, NMR analysis). The reasons for this, as well as for the formation of only the (*E*)-ethylidene lactone, are not obvious from simple inspection of models, and might be somehow connected to the unique conformational features of *VIII*

TABLE I

11,13-Methylenation of sesquiterpene exomethylene-γ-lactones: compounds tested and yields

Compound	Yield[a], %	Eluant used for the chromatographic purification hexane–ethylacetate
Costunolide (*I*)	64	7 : 3
Artemorin (*XVI*)	55	3 : 7
Artemorin acetate (*XVIII*)	65	6 : 4[b]
Parthenolide (*XX*)	51	8 : 2[b]
Tatridin A diacetate (*XXII*)	45	8 : 2[b]
(*Z,E*)-14-hydroxygermacra-1(10)-4,11(13)-trien-*trans*-6-olide (*XXIV*)	68	5 : 5[b]
Dehydrocostuslactone (*XXVI*)	59	95 : 5
3-Epizaluzanin C (*XXVIII*)	53	5 : 5
Graveolide (*XXX*)	47	7 : 3
5-Epitelekin (*XXXII*)	66	4 : 6
Telekin (*XXXIV*)	68	4 : 6[b]
Santamarine (*XXXVI*)	65	4 : 6
Reynosin (*XXXVIII*)	70	4 : 6

[a] Based on the starting exomethylene-γ-lactone. Crude pyrazoline adducts were used for the photolysis without any further purification; [b] HPLC was required to remove small amounts of the ethylidene lactones. The same eluant used for column chromatography was employed.

and its derivatives, that have an essentially flat γ-lactone ring[12] (pseudorotational P-type[17]). In all the other compounds tested, the γ-lactone ring is instead puckered (pseudorotational S- or A-type[17]). Comparison of the ^{13}C NMR spectral features of sesquiterpene cyclopropyl- and exomethylene-γ-lactones showed a marked downfield shift of the lactone carbonyl (c. 10 ppm), that parallels the one observed upon hydrogenation of the exomethylene. As to the ^{1}H NMR spectra, methylenation of the exomethylene caused a downfield shift (c. 0·10 ppm) of the lactone methine (H-6 or H-8, depending on the site of lactonization), which is probably due to the magnetic anisotropy of the cyclopropane ring.
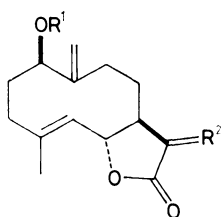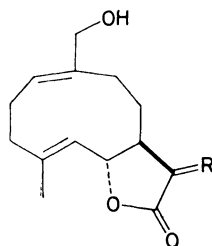

XVI, R¹= H ; R²= =CH₂
XVII, R¹= H ; R²= —CH₂—CH₂—
XVIII, R¹= Ac ; R²= =CH₂
XIX, R¹= Ac ; R²= —CH₂—CH₂—
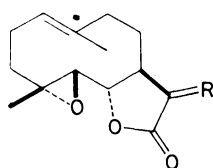
XX, R = =CH₂
XXI, R = —CH₂—CH₂—
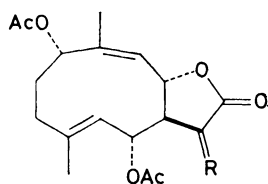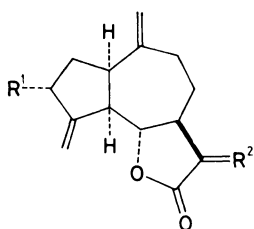
XXII, R = =CH₂
XXIII, R = —CH₂—CH₂—

XXIV, R.= =CH₂
XXV, R = —CH₂—CH₂—

A study of the reactivity of cyclopropyl lactones *III*, *XXI* and *XXXV* towards the bionucleophile cysteine showed that these compounds are completely unreactive in conditions under which their corresponding exomethylene-γ-lactones form Michael adducts (water–ethanol, pH 7·4). No evidence of a reaction could be observed under a variety of other experimental conditions, and the cyclopropyl lactones *XXI* and *XXXV* did not show in fact cytotoxic activity, in contrast to their corresponding exomethylene lactones[18]. However, *III* displayed an "in vitro" cytotoxic activity comparable to that of its corresponding exomethylene lactone costunolide *I*, with an ID$_{50}$ of 0·120 μmol/ml on EVSA-T cells and 0·200 μmol/ml on HL-60

XXVI, R^1= H ; R^2= =CH_2
XXVII, R^1= H ; R^2= —CH_2—CH_2—
XXVIII, R^1= OH ; R^2= =CH_2
XXIX, R^1= OH ; R^2= —CH_2—CH_2—
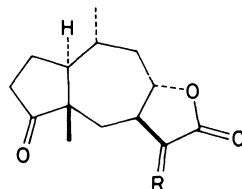
XXX, R = =CH_2
XXXI, R = —CH_2—CH_2—
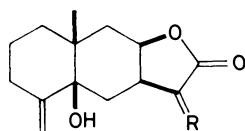
XXXII, R = =CH_2
XXXIII, R = —CH_2—CH_2—

XXXIV, R = =CH_2
XXXV, R = —CH_2—CH_2—
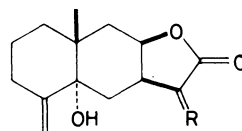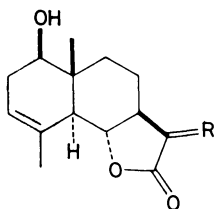
XXXVI, R = =CH_2
XXXVII, R = —CH_2—CH_2—
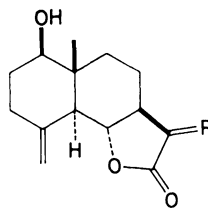
XXXVIII, R = =CH_2
XXXIX, R = —CH_2—CH_2—

cells (the corresponding values for *I* were 0·159 μmol/ml on EVSA-T cells and 0·158 μmol/ml on HL-60 cells[18]). The precise mechanism of this activity is at present not known. On the basis of the above mentioned "in vitro" reactivity studies, alkylation of bionucleophiles might not be involved in it.

EXPERIMENTAL

Melting points were determined on a Büchi SMP 20 apparatus and are uncorrected. Electron-impact mass spectra were taken on a Varian Mat CH7A apparatus. IR spectra were recorded on a Perkin–Elmer model 237 spectrophotometer (wave numbers in cm^{-1}). ^{1}H and ^{13}C NMR spectra were obtained on a Varian VXR 300 spectrometer (300 and 75·2 MHz, respectively). Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. Indirect internal

standardization was applied (δ CHCl$_3$ = 7·24; δ CDCl$_3$ = 77·0). Silica gel 60 (70—230 mesh, Merck) was used for column chromatography. A Waters microporasyl column (80 $\times$ 3 cm) was used for preparative HPLC, using a Waters differential refractometer R 401.

Compounds *I*, *XVI*, *XXXVI* and *XXXVIII* (ref.[19]), *VIII* (ref.[12]), *XXXII* and *XXXIV* (ref.[20]), *XX* (ref.[21]) and *XXX* (ref.[22]) were available from previous work; *XXVI* was isolated from costus oil; *XVIII* and *XXII* were prepared by acetylation of their corresponding alcohols (artemorin and tatridin A) with acetic anhydride–pyridine. *XXIV* and *XXVIII* were prepared by allylic oxidation[23] of costunolide and dehydrocostus lactone (tert-butyl hydroperoxide, selenium(IV) oxide, dichloromethane).

11,13-Methylenecostunolide (*III*)

Under a nitrogen atmosphere, a solution of dimethyloxosulfonium iodide[10] (365 mg, 1·66 mmol 1·2 molar equiv.) in DMSO (3 ml) was added via syringe to sodium hydride (40 mg, 1·66 mmol), used as a 50% suspension in mineral oil, and washed three times with dry pentane. The milky suspension was stirred for 20 min at room temperature, and then a solution of costunolide *I* (300 mg, 1·30 mmol) in DMSO (2·8 ml) was added dropwise. A yellowish solution was formed at the end of the addition, and the reaction was quenched by the adition of saturated aqueous ammonium chloride and exctracted with methylene chloride. The organic phase was dried over anhydrous magnesium sulfate and evaporated. The residue (78 mg) was purified by column chromatography (5 g silica gel, eluant: hexane–ethylacetate 95 : 5) to give 64 mg (20%) of *III*. The aqueous phase from the reaction (pH c. 7·5) was acidified with diluted hydrochloric acid to pH 2, and then extracted with ethylacetate. The solid residue (214 mg) had IR bands at 3 400, 2 910, 2 840, (no carbonyl band), 1 020 and 910, and presented a (pseudo)parent ion in the mass spectra at m/z 361 (9%). The ^{1}H NMR spectrum showed a mixture of products. Compound *III*. M.p. 100°C (hexane–ether), $[\alpha]_D^{25}$ +80° (c 1·8, chloroform). IR spectrum (KBr): 1 750, 1 450, 1 440, 1 340, 1 310, 1 270, 1 210, 1 170, 1 150, 960. ^{1}H NMR spectrum (C$_6$D$_6$): 4·58 bt, 1 H (H-1, J = 5·0); 4·51 d, 1 H (H-5, J = 10·0); 4·32 t, 1 H (H-6, J = 10·0); 1·40 bs, 3 H (H-14), 1·10 bs, 3 H (H-15); 0·80—0·20 m, 4 H (cyclopropane protons). ^{13}C NMR spectrum (CDCl$_3$): 179·40 s (C-12); 140·35, 136·99 s (C-10 and C-4); 127·60, 127·05 d (C-1 and C-5); 82·08 d (C-6); 48·21 d (C-7); 40·75, 39·48 t (C-9 and C-3); 26·13, 25·55 t (C-2 and C-8); 25·04 s (C-11); 17·29, 16·10 q (C-14 and C-15); 11·38, 8·61 t (C-13 and C-16). Mass spectrum, m/z: 246 (C$_{16}$H$_{22}$O$_2$; M$^+$, 21), 231 (14), 189 (9), 165 (20), 145 (14), 121 (42), 109 (59), 81 (100), 67 (42), 41 (91).

Reaction of Umbellifolide (*VIII*) with Dimethyloxosulfonium Methylide

Umbellifolide (*VIII*) (200 mg, 0·75 mmol) was reacted with dimethyloxosulfonium methylide (prepared from 210 mg (1·2 molar equiv.) of dimethyloxosulfonium iodide) as described for costunolide. After working-up the reaction, the residue from the organic phase (89 mg) was separated by HPLC (hexane–ethylacetate 6 : 4) to give 7 mg unreacted *VIII*, 12 mg (5%) *X* and 33 mg (15%) *IX*.

11,13-*Methylenumbellifolide* (IX). M.p. 63° (isopropyl ether), IR spectrum (KBr): 1 760, 1 720, 1 450, 1 420, 1 360, 1 290, 1 250, 1 170, 1 150, 1 110, 740. ^{1}H NMR spectrum (CDCl$_3$): 5·08 td, 1 H (H-8, J = 8·3, 8·3, 5·0); 2·10 s, 3 H (H-15); 1·09 s, 3 H (H-14); 1·20—0·85, 4 H (cyclopropane protons). Mass spectrum, m/z: 278 (C$_{16}$H$_{22}$O$_4$, M$^+$, 15), 221 (40), 194 (90), 84 (75), 43 (100).

11,13-4,O′4)-*Bismethylenumbellifolide* (X). Gum, IR spectrum (liquid film): 1 760, 1 710, 1 360, 1 290, 1 130, 970. ^{1}H NMR spectrum (CDCl$_3$): 4·63 td, 1 H (H-8, J = 4·8, 4·8, 2·9); 2·91 d,

1 H (H-17a, $J = 4\cdot5$); 2·59 d, 1 H (H-17b, $J = 4\cdot5$); 1·39 s, 3 H (H-15); 1·07 s, 3 H (H-14). Mass spectrum, m/z: 292 ($C_{17}H_{24}O_4$, M^+, 1), 276 (1), 221 (70), 194 (80), 84 (100). The minor isomer at C-4 had the following NMR data: 4·69 td, 1 H (H-8, $J = 4\cdot8$, 4·8, 2·9); 2·78 d, 1 H (H-17a, $J = 4\cdot5$); 2·51 d, 1 H (H-17b, $J = 4\cdot5$); 1·42 s, 3 H (H-15); 1·09 s, 3 H (H-14).

Reaction of Costunolide (*I*) with Tribromomethyl Phenyl Mercury

To a stirred solution of *I* (100 mg, 0·43 mmol) in dry benzene (3 ml), tribromomethyl phenyl mercury (212 mg, 0·43 mmol, 1 molar equivalent) was added, and the resulting suspension was refluxed for two hours under nitrogen. After removal of the solvent, the black residue was separated by column chromatography (5 g silica gel, hexane–ethylacetate 9 : 1 as eluant) to give 37 mg (37%) *XII*, 20 mg (20%) *XIII*, and 55 mg (34%) *XI*. Compounds *XII* and *XIII* were identified by comparison of their spectral features (^{1}H NMR) with those of authentic samples.

1,10-*Dibromomethylencostunolide* (XI). M.p. 104—106°C (decomposition), IR spectrum (KBr): 1 750, 1 660, 1 460, 1 320, 1 120, 940, 730, ^{1}H NMR spectrum (CDCl$_3$): 6·29 d, 1 H (H-13a, $J = 3\cdot0$); 5·54 d, 1 H (H-13b, $J = 3\cdot0$); 5·40 dd, 1 H (H-6, $J = 10\cdot9$, 8·0); 5·12 d, 1 H (H-5, $J = 10\cdot9$); 3·10 m, 1 H (H-7); 1·75 s, 3 H (H-15); 1·05 s, 3 H (H-14). Mass spectrum, m/z: 406/404 ($C_{16}H_{20}Br_2O_2$, M^+, 5), 325, 323 (12), 243 (100), 165 (60), 91 (95).

Reaction of Costunolide (*I*) with Diazomethane and Palladium(II) Acetate

To a stirred and cooled (0°C) suspension of costunolide (*I*) (100 mg, 0·43 mol) and palladium(II) acetate (5 mg) in ether (3 ml), an excess ethereal diazomethane was added dropwise using a dropping funnel equipped with a teflon stopcock. After stirring 45 min at 0°C, the excess diazomethane was destroyed by the addition of a teaspoon of silica gel. The slurry was stirred 10 min, untill the evolution of nitrogen had ceased, and then filtered and evaporated. The residue, an oil, was a mixture of products. ^{1}H NMR showed the presence of costunolide (*I*), its pyrazoline *II* as well as several unidentified products. No upfield signals below 1 ppm, characteristic of the cyclopropane protons, were present.

Formation of the Pyrazoline Adducts of Exomethylene-γ-lactones and their Photolysis (Reaction with *I* is Reported as Representative)

To a cooled (0°C) solution of *I* (1 g, 4·3 mmol) in ether–dichloromethane (5 : 1), an excess ethereal diazomethane (C5 molar equiv., generated from Diazald) was added. After standing overnight at 0°C, TLC (hexane–ethylacetate 7 : 3) showed that all starting material had reacted, and the excess diazomethane was destroyed by the addition of silica gel (c. 1 g, column chromatography grade). After stirring 10 min, the slurry was filtered, the residue washed two times with c. 10 ml of CH$_2$Cl$_2$, and the filtrate was evaporated to give a solid residue (995 mg), which was used directly for the next step.

The crude pyrazoline *II* (995 mg, 3·63 mmol) was disolved in dry toluene (distilled from Na--Benzophenone) (c. 100 ml, $c \sim 3\cdot5 \cdot 10^{-2}$ mol l^{-1}). The solution was transferred to an immersion well reactor (Applied Photophysics Ltd), degassed for 10 min bubbling nitrogen into it, and then irradiated with a medium-pressure mercury lamp (400 W, Applied Photophysics Ltd). The reaction was followed by TLC (hexane–ethylacetate 7 : 3). After 2·5 h the reaction was worked-up by evaporating the solvent and the residue was dissolved in methanol (5 ml, 0·5 ml/100 mg of starting pyrazoline). The solution was cooled to 0°C, and diethylamine (1 ml, c. 1 ml/g of starting pyrazoline) was added. The solution was left at room temperature for 3 h, and then

evaporated. The residue was charged on a column prepared as follows: a slurry of 30 g silica gel in hexane–ethylacetate 7 : 3 (c. 3 g silica gel/1 g of starting pyrazoline) was packed into the column; two filters were put at the top of the silica layer, and then a slurry of basic alumina (50 g, c. 5 g basic alumina/1 g of starting pyrazoline) was stratified on the silica gel layer. The column was eluted with hexane–ethylacetate to give 625 mg (70%) *III* and 71 mg (8%) *IV*. The Mannich base was not recovered from the column.

This procedure was applied to the sesquiterpene lactones listed in Table I. In some cases (compounds *XVI*, *XXXII*, *XXXIV*, *XXXVI* and *XXXVIII*) a small amount of dry dioxane (c. 5% (v/v)) was used to increase the solubility of the pyrazoline adduct(s) in the photolytic step. The scale of the reaction (based on the starting exomethylene lactones) was 300 mg, except for *XVI*, *XX*, *XXVI*, *XXXVI* (1 g) and *XVIII* and *XXIV* (500 mg). For the final chromatographic purification of the cyclopropyl lactones, the eluant mixtures listed in Table I were employed.

11,13-*Methylenartemorin* (XVII). Gum, IR spectrum (liquid film): 3 450, 1 760, 1 450, 1 340, 1 280, 1 140, 970, 940. ^{1}H NMR spectrum (CDCl$_3$): 5·24 d, 1 H (H-5, $J = 10·0$); 5·18 bs, 1 H (H-14a); 4·81 bs, 1 H (H-14b); 4·51 t, 1 H (H-6, $J = 10·0$); 3·85 bm, 1 H (H-1); 1·69 bs, 3 H (H-15). Mass spectrum, m/z: 262 (C$_{16}$H$_{22}$O$_3$, M$^+$, 5), 244 (M − 18)$^+$ (25), 229 (18), 163 (80), 109 (100).

11,13-*Methylenartemorin acetate* (XIX). Gum, IR spectrum (liquid film): 1 780, 1 740, 1 460, 1 380, 1 350, 1 290, 1 250, 1 150, 1 060, 1 030, 980, 950, 750. ^{1}H NMR spectrum (CDCl$_3$): 5·35 bd, 1 H (H-5, $J = 10·0$); 5·20 bs, 1 H (H-14a); 4·90 bs, 1 H (H-14b); 4·85 bm, 1 H (H-1); 4·45 t, 1 H (H-6, $J = 10·0$); 1·98 s, 3 H (acetate); 1·65 bs, 3 H (H-15); 1·30—0·70 m, 4 H (cyclopropane protons). ^{13}C NMR spectrum (CDCl$_3$): 179·50 s, (C-12); 170·24 s (acetate); 147·44, 144·72 s (C-10 and C-4); 122·90 d, (C-5); 112·25 t (C-14); 81·15 d (C-6); 78·85 d (C-1); 47·55 d (C-7); 36·68, 31·10, 31·07, 25·28 t (C-9, C-2, C-8 and C-3); 24·79 s (C-11); 21·30 s (acetate); 17·88 q (C-15); 11·06, 8·52 t (C-13 and C-16). Mass spectrum, m/z: 304 (C$_{18}$H$_{24}$O$_4$, M$^+$, 5), 262 (M − 42, 10), 244 (M − AcOH, 75), 229 (15), 43 (100).

11,13-*Methylenparthenolide* (XXI). M.p. 182°C (ether), [α]$_D^{25}$ −76·7° (c 1·1, chloroform). IR spectrum (KBr): 1 770, 1 460, 1 440, 1 340, 1 280, 1 210, 1 140, 1 080, 990, 820. ^{1}H NMR spectrum (CDCl$_3$): 5·18 dd, 1 H (H-1, $J = 11·9, 3·8$); 3·94 t, 1 H (H-6, $J = 9·2$); 2·82 d, 1 H (H-5, $J = 9·2$); 1·68 bs, 3 H (H-14); 1·31 s, 3 H (H-15); 1·30—0·70 m, 4 H (cyclopropane protons). Mass spectrum, m/z: 262 (C$_{16}$H$_{22}$O$_3$, M$^+$, 4), 244 (5), 204 (30), 93 (40), 86 (100).

11,13-*Methylentatridin A diacetate* (XXIII). M.p. 185°C (hexane–ether), [α]$_D^{25}$ −46° (c 0·5, chloroform). IR spectrum (KBr): 1 770, 1 740, 1 450, 1 420, 1 370, 1 270, 1 240, 1 140, 1 020, 990, 960. ^{1}H NMR spectrum (C$_6$D$_6$): 5·57 t, 1 H (H-1, $J = 6·5$); 5·18 t, 1 H (H-8, $J = 10$); 5·04 d, 1 H (H-5, $J = 10$); 4·84 t, 1 H (H-6, $J = 10·0$); 4·54 bd, 1 H (H-9, $J = 10$); 2·34 t, 1 H (H-7, $J = 10·0$); 1·90 bs, 3 H (H-15); 1·60 s, 1·49 s, 2 × 3 H (acetates), 1·40—0·70 m, 4 H (cyclopropane protons). Mass spectrum, m/z: 362 (C$_{20}$H$_{26}$O$_6$, M$^+$, 20), 303 (25), 260 (30), 243 (25), 227 (18), 43 (100).

11,13-*Methylen-(Z, E)-14-hydroxygermacra*-1(10),4,11(13)-*trien-trans-6-olide* (XXXV). Gum, [α]$_D^{25}$ +5·7° (c 2·7, chloroform). IR spectrum (liquid film): 3 450, 1 770, 1 670, 1 450, 1 340, 1 280, 1 140, 980, 870. ^{1}H NMR spectrum (CDCl$_3$): 5·46 t, 1 H (H-1, $J = 7·5$); 5·05 d, 1 H (H-5, $J = 10·2$); 4·74 t, 1 H (H-6, $J = 10·2$); 4·00 m, 2 H (H-14a and H-14b); 1·86 bs, 3 H (H-15); 1·30—0·70 m, 4 H (cyclopropane protons). ^{13}C NMR spectrum (CDCl$_3$): 180·12 s (C-12); 140·94. 137·40 s (C-10 and C-4); 125·96, 125·87 d (C-1 and C-5); 81·47 d (C-6); 66·03 t (C-14); 42·84 d (C-7); 38·46 t (C-3); 25·10 s (C-11); 24·79, 23·61, 23·21 t (C-2, C-8 and C-9); 17·13 q (C-15); 11·57, 9·40 t (C-13 and C-16). Mass spectrum, m/z: 262 (C$_{16}$H$_{22}$O$_3$, M$^+$, 30), 244 (45), 204 (40), 164 (80), 105 (70), 91 (80), 81 (100).

11,13-*Methylendehydrocostuslactone* (XXVII). M.p. 69°C (hexane), $[\alpha]_D^{25}$ +30° (c 2·1, chloroform). IR spectrum (KBr): 1 760, 1 650, 1 460, 1 440 , 1 420, 1 350, 1 320, 1 270, 1 230, 1 150, 1 130, 990, 890. ^{1}H NMR spectrum (CDCl$_3$): 5·23 bs, 1 H (H-15a); 5·04 bs, 1 H (H-15b); 4·85 bs, 1 H (H-14a); 4·75 bs, 1 H (H-14b); 4·03 t, 1 H (H-6, J = 9·5); 1·30—0·70 m, 4 H (cyclopropane protons). Mass spectrum, m/z: 244 (C$_{15}$H$_{20}$O$_2$,M$^+$, 100), 228 (45), 164 (95), 117 (75), 91 (90), 79 (80).

11,13-*Methylen-3-epizaluzanin C* (XXIX). M.p., 110°C (hexane–ether), $[\alpha]_D^{25}$ —16° (c 1·1, chloroform). IR spectrum (KBr): 3 420, 1 760, 1 670, 1 640, 1 350, 1 150, 1 010, 985, 905, 735. ^{1}H NMR (CDCl$_3$): 5·49 bs, 5·35 bs, 4·90 bs, 4·75 bs, 4 × 1 H (H-14a, H-14b, H-15a, and H-15b); 4·71 bt, 1 H (H-3, J = 6·0); 4·00 t, 1 H (H-6, J = 9·5); 1·25—0·70 m, 4 H (cyclopropane protons). Mass spectrum, m/z: 260 (C$_{16}$H$_{20}$O$_3$, M$^+$, 45), 242 (75), 164 (100), 91 (70).

11,13-*Methylengraveolide* (XXXI). M.p. 120°C (hexane–ether), $[\alpha]_D^{25}$ +90° (c 1·1, chloroform). IR spectrum (KBr): 1 770, 1 730, 1 345, 1 155, 1 120, 1 005, 990. ^{1}H NMR spectrum (CDCl$_3$): 4·35 ddd, 1 H (H-8, J = 9·8, 4·2, 3·2); 1·06 d, 3 H (H-14, J = 6·5); 1·00 s, 3 H (H-15); 1·10 to 0·70 m, 4 H (cyclopropane protons). Mass spectrum, m/z: 262 (C$_{16}$H$_{22}$O$_3$, M$^+$, 60), 234 (40), 207 (15), 187 (90), 121 (100).

11,13-*Methylen-5-epitelekin* (XXXIII). M.p. 168°C (hexane–ether), $[\alpha]_D^{25}$ +23° (c 1·1, dichloromethane). IR spectrum (KBr): 3 500, 1 750, 1 360, 1 300, 1 170, 1 130, 1 100, 1 040, 960, 930. ^{1}H NMR (CDCl$_3$): 4·90 bs, 1 H (H-15a); 4·88 bs, 1 H (H-15b); 4·79 q, 1 H (H-8, J = 6·4); 1·04 s, 3 H (H-14); 1·20—0·80 m, 4 H (cyclopropane protons). Mass spectrum, m/z: 262 (C$_{16}$H$_{22}$.O$_3$, M$^+$, 100), 234 (40), 194 (35), 124 (85), 95 (80), 67 (75).

11,13-*Methylentelekin* (XXXV). M.p. 189°C, $[\alpha]_D^{25}$ +112° (c 0·62, chloroform). IR spectrum (KBr): 3 500, 1 750, 1 370, 1 310, 1 290, 1 250, 1 220, 1 180, 1 140, 1 120, 1 060, 1 040, 1 010, 960. ^{1}H NMR spectrum (CDCl$_3$): 4·84 bs, 1 H (H-15a); 4·74 dt, 1 H (H-8, J = 4·8, 4·8 and 2·0); 4·71 br, 1 H (H-15b); 0·96 s, 3 H (H-14); 1·25—0·85 m, 4 H (cyclopropane protons). ^{13}C NMR spectrum (CDCl$_3$): 180·08 s (C-12); 150·48 s (C-4); 108·76 t (C-15); 81·07 s (C-5); 77·87 d (C-8); 37·95 d (C-7); 36·85 s (C-10); 35·90, 35·31, 31·70, 31·56, 21·60 t (C-1, C-2, C-3, C-6, and C-9); 28·37 s (C-11); 21·78 q (C-14); 15·84, 7·99 t (C-13 and C-16).

11,13-*Methylensantamarine* (XXXVII). M.p. 89°C, $[\alpha]_D^{25}$ +100° (c 1·1, chloroform). IR spectrum (KBr): 3 300, 1 780, 1 440, 1 380, 1 350, 1 280, 1 140, 1 030, 990, 940. ^{1}H NMR spectrum (CDCl$_3$): 5·33 bs, 1 H (H-3); 4·11 t, 1 H (H-6, J = 9·6); 3·67 m, 1 H (H-1); 1·84 bs, 3 H (H-15); 0·89 s, 3 H (H-14); 1·35—0·75 m, 4 H (cyclopropane protons). Mass spectrum, m/z: 262 (C$_{16}$H$_{22}$O$_3$, M$^+$, 100), 244 (20), 177 (40), 166 (70), 121 (30), 107 (30), 91 (35).

11,13-*Methylenreynosin* (XXXIX). M.p. 104°C (acetone–ether), $[\alpha]_D^{25}$ +105° (c 0·92, chloroform). IR spectrum (KBr): 3 480, 1 760, 1 650, 1 450, 1 350, 1 270, 1 140, 1 000, 960. ^{1}H NMR spectrum (CDCl$_3$): 4·98 bs, 1 H (H-15a); 4·88 bs, 1 H (H-15b); 4·19 t, 1 H (H-6, J = 11·0); 3·51 dt, 1 H (H-1, J = 11·6, 3·5, 3·5); 0·81 s, 3 H (H-14); 1·35—0·75 m, 4 H (cyclopropane protons). Mass spectrum, m/z: 262 (C$_{16}$H$_{22}$O$_3$, M$^+$, 25), 244 (100), 177 (90), 119 (25), 107 (25), 91 (30), 79 (35).

Reaction of 11,13-Methylencostunolide (*III*) with Cysteine

To a solution of L-cysteine hydrochloride hydrate (33 mg, 0·21 mmol) in 1 : 1 degassed ethanol–phosphate buffer (pH 7·4, 2 ml), a degassed ethanolic solution of *III* (54 mg, 0·21 mmol, 0·5 ml) was added via syringe, and the solution was stirred at room temperature. The reaction was controlled by TLC for one month, but no reaction occurred. When costunolide (*I*) was reacted under the same conditions, a precipitate started to appear after 8 h, and the reaction was com-

plete (TLC control) after two days. The Michael adduct[24] was filtered and dried in vacuo (yield: 35%). No evidence of reaction was obtained when *III* was treated with cysteine in DMF, ether––water or THF–water. Analogous results were also obtained with the cyclopropyl lactones *XXI* and *XXXV*.

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