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CHEMISTRY OF COSTUNOLIDE

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Abstract—Costunolide (I) on reaction with diazomethane affords a pyrazoline derivative (II) whose pyrolysis furnishes three products. The structures of all these compounds have been completely established by spectral methods and chemical correlation. In this study fourteen costunolide derivatives have been prepared and these have been found active as plant growth regulators.

Plant growth activity of several α-methylene-γ-lactones has been well documented.[1] It has been established that this activity is due to the exo-methylene group conjugated to the lactone CO and this structural feature is almost indispensable[2] for this action. It has also been shown that some γ-lactones in which a trisubstituted double bond or a cyclopropane ring is conjugated with the lactone carbonyl are even more active[3] than the parent α-methylene- γ-lactones. Significantly, cross conjugated ketone moiety has been recently reported to promote growth in plants.[4]

In order to provide an opportunity to establish whether the plant growth activity of γ-lactones is due to double bond conjugation in the α,β-position and to obtain further structure-activity data we set out to synthesize several derivatives from costunolide. The present communication reports the synthesis of fourteen compounds. Their evaluation as plant growth regulators has confirmed the structure-activity correlation reported earlier from our laboratory.[3] These biological data will be published separately.

Costunolide (I) on reaction with diazomethane affords a crystalline adduct $C_{16}H_{22}O_2N_2$, m.p. 150°, M^+ 274. The IR spectrum showed the absence of band at 820 cm^{-1} typical[5] of α-methylene in conjugation with lactone moiety. The presence of γ-lactose ($\gamma^{C=O}$ 1760, —N═N—, 1560 cm^{-1}) and the NMR spectrum (Table I) were sufficient to characterize this compound as the pyrazoline derivative (II, without stereochemistry).

The pyrazoline on pryolysis affords a three component mixture which on chromatographic separation gave three crystalline compounds m.p. 99°, 140° and 120°.

The compound m.p. 99°, $C_{16}H_{22}O_2$ displayed an intact γ-lactone and showed the absence of band due to —N═N—. Its NMR spectrum clearly showed the presence of cyclopropane protons, this coupled with the other NMR features suggested structure III for this compound. This structure was further confirmed by the chemical correlation reported later.

The compound m.p. 140°, $C_{16}H_{22}O_2$ contained a γ-lactone moiety. Its NMR features initially led us to believe that it was a mixture of E and Z isomers (IV and V respectively), however, a comparison of the NMR spectrum with that of costunolide (Table I) clearly showed that the E isomer is only present.

Thus in costunolide the protons of the α-methylene group appear as two pairs of dd one of which is located at 5.47 and the other 6.28. Literature reveals[6] that the proton having a *cis* relation to the CO is usually deshielded due to its eclipsing relation with the CO. As our compound shows its C_{13} olefinic proton at 6.07, it obviously must have this hydrogen *cis* to the CO and hence must be E isomer.† Its appearance as a doublet of quartet shows its coupling with C_{13}Me and C_7 allylic H. Significantly this latter coupling is large (2Hz). More significantly the C_{16}H (Me at C_{13}) also appear as double doublets due to coupling with C_{13}H and a large homoallylic coupling (2Hz). Similar cases of large allylic and homallylic coupling are recorded in literature.[7] This argument was also confirmed by examining the 60 and 90 MHz spectra of this compound. Based on these arguments compound m.p. 140° was assigned structure IV.

The compound m.p. 120° displayed typical features for methylenic and vinyl double bonds (bands at 1650, 890, 920 and 1010 cm^{-1}) which were absent in the other derivatives. This itself was a sufficient evidence to suggest a transformation of the type dihydrocostunolide (VI) to Sausurrea lactone[8] (VII). These IR spectral features were in conformity with the NMR spectrum which further displayed a doublet of quartets at 6.13 typical of E isomer mentioned above. This suggested structure VIII for this compound.

Chemical correlation. In order to show that pyrolysis had not affected the thermally labile 10-membered ring of costunolide present in the compounds m.p. 99° and 140° we carried out a chemical correlation of these compounds based on the known cyclization[5] of costunolide to cyclocostunolides.

Compound m.p. 99° on reaction with BF_3-etherate gave a mixture which on chromatographic

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† In one of our experiments we were able to isolate a product whose NMR spectrum revealed it to be a mixture of E and Z isomers. Thus it clearly showed a second multiplet at 5.30 and in the allylic methyl region a more complex pattern due to the presence of both isomers was observed.

Table 1. NMR spectra of costunolide derivatives (chemical shift in δ ppm)

Compound	C_4 & C_{10} Methyls	C_6H	$C_{16}H$	C_1 & C_5H	$C_{13}H$
II	1.43, 3H bs 1.83, 3H bs	5.53, dd J = 9, 10 Hz	4.85, 4H complex multiplet		—
III	1.40, 3H bs 1.73, 3H bs	4.8 m hidden by C_1 and C_5H	0.8 & 1.2 m	4.8 m	0.8–1.2 m
IV	1.4, 3H bs 1.7, 3H bs	4.4 t J = 9.5 Hz	2.13, dd 3H J = 7.5, 2 Hz	4.63 m	6.07, dq J = 7.5, 2 Hz
VIII	1.81, 3 H bs 1.07, 3H s	4.13 t J = 11.5 Hz	2.2, dd 3H	C_1H, 5.83 m C_2 & C_3H, 5.0 m 4H	6.13, dq 1H J = 3, 7 Hz
I	1.4, 3H bs 1.66, 3H bs	4.5 t J = 11 Hz	—	4.75–5.08 m	5.47 d J = 3.5 Hz 6.2 d J = 3.5 1 H each

Table I. (*Contd*)

Compound	$C_{10}Me$	C_6H	$C_{13}H$	$C_{16}H$	$C_{15}H$
X	0.82 s	3.95, t J = 11.0 Hz	5.35, 1H d J = 3 Hz 6.03, 1H d J = 3 Hz	—	4.75, 1H bs 4.90, 1H bs
XIIa	0.93 s	5.23, t J–11 Hz	—	4.32, 2 H t J = 8 Hz	4.95, 1H bs 5.04, 1H bs
XIIb	0.89 s	4.18, t J = 11 Hz	—	4.69, 2 Hm	4.8, 1 H bs 5.0, 1 H bs
IX	0.9 s	4.25, t J = 11 Hz	0.8–1.1 m	0.8–1.1 m	4.95, 1H bs 5.07, 1H bs
XIII	0.85 s	3.97, t J = 11 Hz	6.0, 1H m	2.13, 3H dd J = 2, 7 Hz	4.85, 1H bs 5.0, 1H bs

Table I. (*Contd*)

Compound	$C_{10}Me$	C_6H	$C_{13}H$	$C_{16}H$	C_3H	C_4Me
XIV	0.9 s	3.85, t J = 11 Hz	5.37, 1H d J = 3 Hz 6.02, 1 H d J = 3 Hz	—	5.37 m	1.8 bs
XV	1.0 s	5.1, t J = 11 Hz	—	4.69, 2 H t J = 8.5 Hz	5.42 m	1.92 bs
XVI	1.2 s	5.78, d J = 11 Hz	—	4.78, 2 H t J = 8 Hz	—	1.98 bs
XVII	0.97 s	4.17, t J = 11 Hz	0.8–1.2 m	0.8–1.2 m	5.5 nm	1.9 bs
XVIII	0.9 s	3.9, t J = 11 Hz	6.1, 1 H dq J = 8, 3 Hz	2.17, 3 H dd J = 3, 8 Hz	5.5 nm	1.87 bs

separation furnished the major product as a crystalline compound m.p. 78°. From its IR and NMR (Table I) features this compound was assigned structure IX. This compound could also be obtained from β-cyclocostunolide (X) of known structure and stereochemistry.[5] β-cyclocostunolide on reaction with diazomethane affords a mixture of two products. From this mixture two pyrazolines m.p. 101° (major) and 195° (minor) were obtained. The IR spectra of these two isomers clearly rule out the possibility of tautomerization as neither isomer has any band to —NH group. The alternative mode of addition of diazomethane to afford XI is ruled out from the NMR spectrum. Thus, from their spectral features these compounds are obviously stereoisomers at C_{11}.

Significantly, in one of the isomers m.p. 101° the C_6 proton is appreciably deshielded and appears at 5.23 whereas in the other it appears at 4.18. Based on the known deshielding of the —N═N— grouping it is obvious that compound m.p. 101° must be represented by XIIa whereas the other pyrazoline must be represented by XIIb. Examination of models shows that XIIb will be more difficult to form than XIIa since in XIIb there is non-bonded interaction of C_{13} methylene with C_6 and C_8 protons. In XIIa on the other hand these interactions are of much smaller magnitude.

Pyrolysis of compound m.p. 101° furnishes two isomeric products m.p. 78° and 92°. The spectral features of these compounds are in complete accord with structure IX and XIII respectively. The compound m.p. 78° is identical in every respect (tlc, mmp, IR) with the compound obtained by the BF_3 cyclization of compound m.p. 99. This suggested that in compound m.p. 99° the 10 membered carbocyclic ring is intact.

Compound IV m.p. 140° had also an intact 10-membered carbocyclic ring as shown by its cyclization with BF_3-etherate which afforded a compound m.p. 92° identical in every respect with the compound XIII prepared from β-cyclocostunolide.

For the synthesis of more compounds to establish structure-reactivity relationship, α-cyclocostunolide (XIV) was made to undergo reaction carried out for β-cyclocostunolide. In this two pyrazolines m.p. 97° and 155° could be obtained. The NMR spectra showed that compound m.p. 97° should be represented by XV without stereochemistry). In compound m.p. 155° the NMR showed C_6 proton as a doublet and shows the absence of C_3 proton. Hence this pyrazoline should be represented by structures XVI. This also explains the low field position of the C_6 proton as compared compounds II, XIIa and XV.

The pyrazoline (XV) m.p. 97° was transformed by pyrolysis into a solid m.p. 73° and a liquid isomer. These were assigned structure XVII and XVIII respectively by an examination of their IR and NMR spectra.

Examination of Table I showed that C_6 proton is appreciably deshielded when costunolide, α and β-cyclocostunolides are converted into pyrozolines (II, XIIa and XV). Based on this large deshielding all these pyrazolines must have the same stereochemistry at C_{11} with the —N═N— grouping β-oriented.

EXPERIMENTAL

General methods. The general isolation methods for chemical reactions consisted of addition of cold water, extraction with ether, washing the extracts with water and drying over Na_2SO_4. Products were isolated by solvent removal. IR spectra were taken in nujol suspensions. NMR spectra were recorded in $CDCl_3$ with TMS as the internal standard. All values are recorded in δ.

Costunolide (I) was isolated from costus roots *Saussurea lappa* Clarke by established methods. It was converted into α- and β-cyclocostunolides as already reported in literature.[3]

Column chromatography over $SiO_2/AgNO_3$ was carried out using the method[9] of Sukh Dev. Tlc was carried out using SiO$_2$/G supplied by BDH. Visualization of spots was done by spraying with H_2SO_4/MeOH followed by heating at 120°.

Pyrazoline (II) *from costunolide* (I). A soln of costunolide (5 g) in ether (100 ml) was allowed to react with an ethereal soln of diazomethane till the yellow colour presisted for ½ hr at 28°. This furnished the adduct m.p. 150° (5.2 g), purified by filtration through SiO_2. (M^+ 274 Found: C, 70.85; H, 9.2. $C_{16}H_{22}O_2N_2$ requires: C, 70.04; H, 8.08%). IR bands at: 1760, 1660 and 1560 cm^{-1}.

Pyrolysis of pyrazoline (II). The pyrazoline (4 g) was heated at its m.p. 150° for 4 hr. The product mixture was chromatographed to furnish compound m.p. 120° in pet ether: ether (5%). (Found: C, 76.84; H, 8.97. $C_{16}H_{22}O_2$. requires: C, 78.01; H, 9.00%). IR bands at: 1750, 1650, 1660, 1010, 920 and 890 cm^{-1}. A compound m.p. 99° was eluted in pet ether: ether (15%). (Found: C, 77.58; H, 9.13. $C_{16}H_{22}O_2$ requires: C, 78.01; H, 9.00%). IR bands at: 1755, 1670 and 830^{-1}. A compound m.p. 140° was eluted in the same solvent system. (Found: C, 77.91; H, 9.20. $C_{16}H_{22}O_2$ requires: C, 78.01; H, 9.00%). IR bands at: 1750, 1650 and 850 cm^{-1}.

Cyclization of compounds m.p. 99° and 140°. The compound 99° (1.0 g) in dry ether (10 ml) was reacted with BF_3-etherate (0.5 ml) at rt for 4 hr. The major product (IX, m.p. 78°) was obtained in pet ether: ether (50%) eluate. (Found: C, 78.21; H, 9.13. $C_{16}H_{22}O_2$ requires: C, 78.01; H, 9.00%). IR bands at: 3070, 1775, 1650 and 892 cm^{-1}.

Similar reaction on compound m.p. 140° afforded a compound XIII, *m.p.* 92°. (Found: C, 78.23: H, 9.11. $C_{16}H_{22}O_2$. requires: C, 78.01, H, 9.00%). IR bands at: 3050, 1750, 1675, 895 and 865 cm^{-1}.

Chemical correlation of compounds IX and XIII with β-cyclocostunolide (X). β-cyclocostunolide (2.0 g) was reacted with diazomethane as above to furnish a major pyrazoline (XIIa, m.p. 101°) in pet ether: ether (25%) eluate. (Found: C, 70.65, H. 9.1. $C_{16}H_{22}O_2N_2$ requires: C, 70.04, H, 8.08%). IR bands at: 3080, 1785, 1645, 1550 and 892cm^{-1}.

The minor pyrazoline (XIIb, m.p. 195° was obtained in pet ether: ether (50%) eluate. (Found: C, 70.45, H, 9.5. $C_{16}H_{22}O_2N_2$ requires: C, 70.04, H, 8.08%). IR bands at: 1775, 1650, 1550 and 892 cm^{-1}.

Pyrolysis of compound XIIa. The pyrolysis as reported above at 110° afforded a mixture. Compound IX m.p. 78° was eluted in pet ether: ether (10%) and compound XIII, m.p. 92° in pet ether: ether (15%). These compounds were found identical (tlc, mmp and IR) with compounds obtained from the BF_3 cyclization of compounds III and IV respectively.

Reaction of diazomethane with α-cyclocostunolide (*XIV*). α-cyclocostunolide on reaction with diazomethane furnished a mixture of pyrazolines. A compound XV m.p. 97° was eluted with pet ether: ether (40%). (Found C: 70.85, H, 9.2. $C_{16}H_{22}O_2N_2$ requires: C, 70.04; H, 8.08%). IR bands at: 1790, 1550 and 840 cm^{-1}.

The minor pyrazoline (XVI, m.p. 155°) was also eluted in pet ether: ether (40%). (Found: C, 70.84, H, 9.19. $C_{16}H_{22}O_2N_2$ requires: C, 70.04, H, 8.08%). IR bands at: 1780, 1550 and 890 cm^{-1}.

Pyrolysis of compound XV, m.p. 97°. Pyrolysis of XV at 100° afforded a mixture of products. A compound XVIII was eluted in pet ether; ether (10%). (Found: C, 77.68; H, 9.06. $C_{16}H_{22}O_2$ requires: C, 78.01; H, 9.0%), while pet ether: ether (15%) eluted a compound XVII, m.p. 73°. (Found: C, 77.75; H, 8.92. $C_{16}H_{22}O_2$ requires: C, 78.01; H, 9.0%). IR bands at: 1780 and 850 cm^{-1}.

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