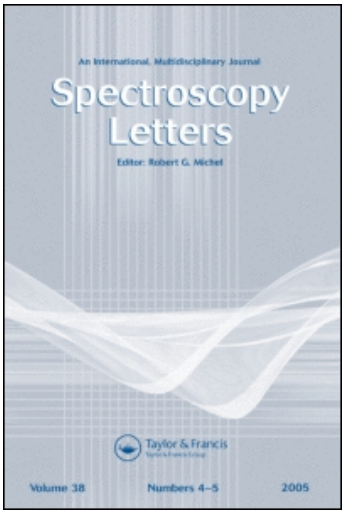

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Spectral Data of Chemical Modification Products of Costunolide

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SPECTRAL DATA OF CHEMICAL MODIFICATION PRODUCTS OF COSTUNOLIDE

Key Words: Sesquiterpene lactones; germacranolides; costunolide; epoxidation-cyclization; parthenolide; 1,10-epoxycostunolide; eudesmanolides; reductions, ^{1}H and ^{13}C NMR.

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Abstract- Epoxidation of costunolide (**1**) yielded parthenolide (**3**), 1,10-epoxycostunolide (**4**), and the cyclization products of **4**, santamarin (**5**), reynosin (**6**), magnolialide (**7**) and a 1,4-epoxyeudesmanolide (**8**). Reduction of santamarin (**5**) with sodium borohydride afforded 11,13-dihydrosantamarin (**9**) and an eudesmen-triol (**10**). Reduction of reynosin (**6**) provided 11,13-dihydroreynosin (**11**) and the two eudesman-triols **12** and **13**. The structures of the new compounds were elucidated by 1D and 2D ^{1}H and ^{13}C NMR spectral methods.

INTRODUCTION

As part of our interest in the biogenesis of various skeletal types of sesquiterpene lactones [1-3], and their biological activities [3-5], we have recently reported on the biomimetic transformations of parthenolide (**3**) [3] and 11βH,13-dihydroparthenolide [2].

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Costunolide (**1**) is proposed to be a key intermediate in the formation of parthenolide, and its guaianolide-type cyclization products [2, 3] as well as 1,10-epoxycostunolide and the derived eudesmanolides [1]. Although epoxidation and cyclization of costunolide (**1**) with peracetic acid and *m*-chloroperbenzoic acid have been described before [6-9], previous studies only reported the epoxidation at the 1,10-position to form 1,10-epoxycostunolide (**4**) [7]. It had been previously shown that cyclizations of **4** produced santamarin (**5**) and reynosin (**6**) [8, 9] and magnolialide (**7**) [9]. However, the 4,5-epoxidation product, parthenolide (**3**), and the eudesmanolide ether **8** were previously not found as reaction products. Our results of the chemical modifications of costunolide and its derivatives are described in this paper.

RESULTS AND DISCUSSION

A solution of costunolide (**1**) in $CHCl_3$ was allowed to react at room temperature with *m*-chloroperbenzoic acid for one hour in the presence of powdered sodium acetate as a buffer. Separation of the reaction mixture by vacuum liquid chromatographic (VLC) [10] on silica gel provided, besides 1,10-epoxycostunolide (**4**) and parthenolide (**3**), the known eudesmanolides santamarin (**5**), reynosin (**6**) and magnolialide (**7**), plus the new 1,4-epoxy-11(13)-eudesmen-12,6-olide (**8**). 1,10-Epoxycostunolide (**4**), santamarin (**5**) and reynosin (**6**) were identified by direct comparison with the spectroscopic data (^1H NMR and MS) of authentic samples and data reported in the literature [6]. Magnolialide (**7**), which had been previously isolated from the root bark of *Magnolia grandiflora* [9], exhibited ^1H NMR and mass spectral data which were essentially identical with those previously reported [9].

Parthenolide (**3**) had been previously isolated from *Magnolia grandiflora* [3] and its structure was established by spectral comparison with the mass spectral and ^1H NMR data of an authentic sample. This is the first report of obtaining parthenolide as an epoxidation product from costunolide (**1**), supporting the proposal that costunolide is the biosynthetic precursor for parthenolide (**3**) [1].

Table 1. ^{1}H NMR spectral data of compounds **8**, **10**, **12**, **13** (400 MHz, $CDCl_3$ as internal standard)

H	8	10	12	13
1	4.00 dd	3.58 dd	3.45 dd	3.43 dd
2α		1.92 m	1.57 m	1.58 m
2β		2.40 m	1.87 m	1.85 m
3		5.33 m	2.07 br dd 2.34 ddd	2.06 m 2.34 ddd
5	1.69 d	1.92 d	1.81 br d	1.78 br d
6	4.00 dd	3.72 dd	3.83 dd	3.77 dd
7	2.37 m	2.08 m	2.14 m	1.56 m
8		1.69 m	1.58 m 1.67 m	1.28 m 1.62 m
9α		1.16 m	1.25 ddd	1.23 m
9β		1.95 m	1.95 ddd	1.90 m
11		------	-------	2.07 m
12		4.13 d 4.17 d	4.11 d 4.16 d	3.51 dd 3.65 dd
13	5.34 d 6.02 d	5.10 br s 5.24 br s	5.09 br s 5.33 br s	0.93 d
14	1.11 s	0.84 s	0.79 s	0.71 s
15	1.55 s	1.87 br s	4.74 br s 5.03 br s	4.74 br s 5.03 br s

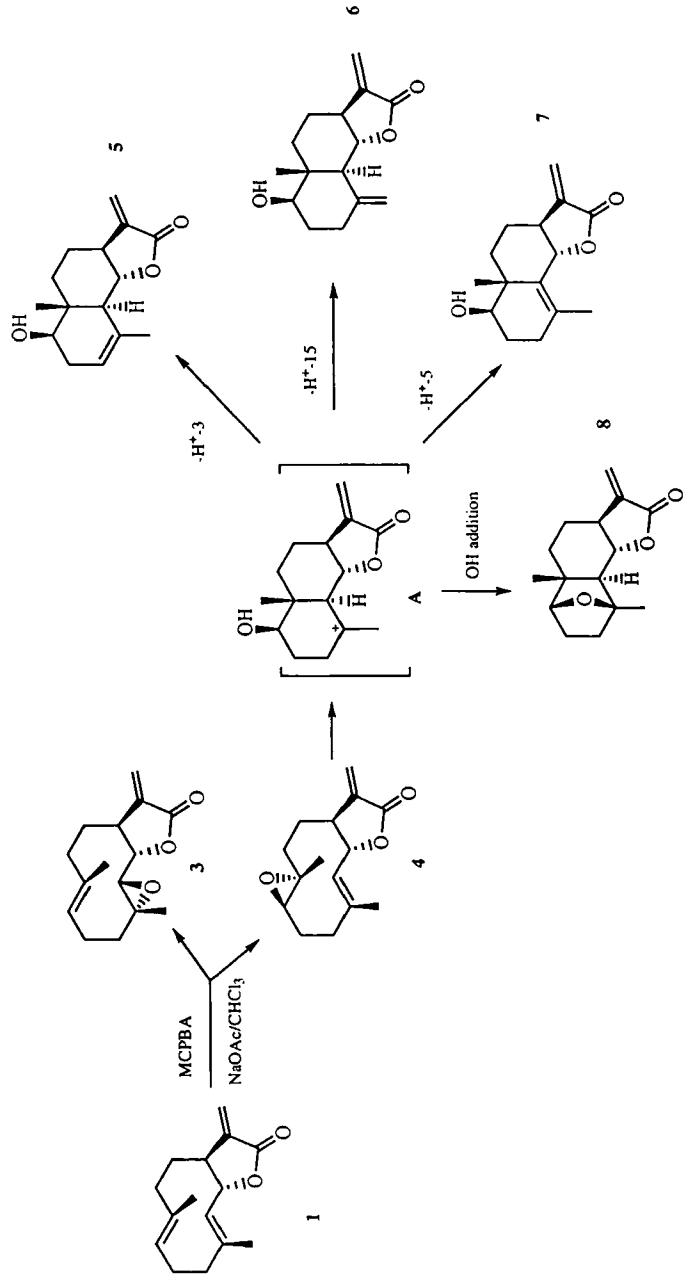
J (Hz): **8**: 1,2α = 6.0, 1,2β = 5.3, 5,6 = 11.2, 6,7 = 10.4, 13a,13b = 3.4; **10**: 1,2α = 7.0, 1,2β = 9.5, 5,6 = 6,7 = 10.0, 12a,12b = 12.6; **12**: 1,2α = 4.7, 1,2β = 11.5, 2α,3α = 5.6, 2α,3β = 4.9, 2β,3β = 2.1, 3α,3β = 13.5, 5,6 = 6,7 = 10.0, 9α,8α = 5.5, 9α,8β = 12.5, 9β,8α = 9β,8β = 3.2, 9α,9β = 13.0, 12a,12b = 12.6; **13**: 1,2α = 4.7, 1,2β = 11.5, 3β, 2α = 4.9, 3β,2β = .2.2, 3α,3β = 13.2, 5,6 =6,7 = 9.8, 12a,11 = 7.5, 12b,11 = 5.4, 12a,12b = 11.2.

The spectral data of compound **8** suggested an eudesmane skeleton very similar to those of santamarin (**5**) and reynosin (**6**). The strong IR absorption at 1772 cm^{-1} indicated the presence of an α,β-unsaturated-γ-lactone. This was further supported by its ^{1}H NMR spectrum which showed the typical lactonic α-methylene doublets at δ 5.34 and 6.02 (Table 1). The ^{13}C NMR spectrum of **8** indicated the presence of 15 carbons including one carbonyl, two CH_3, five CH_2 including one exocyclic methylene, four CH, two being oxygenated, and three quaternary carbons including one olefinic and one oxygenated carbon. The assignments of carbon signals were made by combined application of COSY and DEPT methods and direct comparison with values of related compounds (Table 2). The ^{13}C NMR spectrum of **8** supported the presence of three oxygenated carbons with two methine signals at δ 80.3 and 84.1, and one quaternary carbon signal at δ 83.4, suggesting either a 1,4-diol or 1,4-epoxide moiety in the molecule. Since no hydroxyl

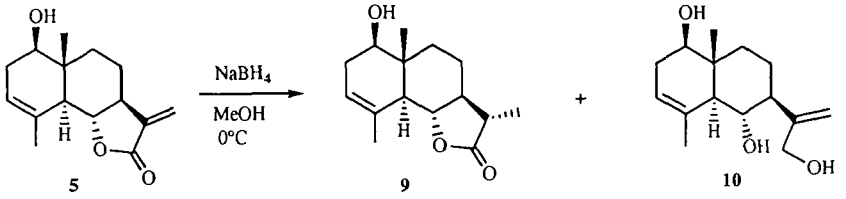
Table 2. ^{13}C NMR spectral data of compounds **8**, **10**, **12** and **13** (100 MHz, $CDCl_3$ as internal standard)*

C	**8**	**10**	**12**	**13**
1	84.1 d	76.0 d	78.8 d	78.9 d
2	42.3 t	32.9 t	31.8 d	31.8 t
3	23.5 t	121.8 d	34.9 t	35.0 t
4	83.4 s	135.2 s	145.3 s	146.0 s
5	59.0 d	52.0 d	55.3 d	55.8 d
6	80.3 d	71.2 d	70.1 d	67.4 d
7	54.0 d	52.0 d	48.7 d	44.8 d
8	30.3 t	26.9 t	26.6 t	20.6 d
9	22.4 t	34.7 t	36.4 t	36.1 t
10	41.8 s	39.7 s	41.8 s	41.5 s
11	138.8 s	151.0 s	151.3 s	36.4 d
12	170.6 s	65.7 t	66.5 t	66.8 t
13	115.8 t	113.0 t	112.5 t	12.9 q
14	17.4 q	10.8 q	11.6 q	11.5 q
15	21.0 q	24.4 q	108.3 t	107.9 t

* Peak multiplicities were determined by heteronuclear multipulse programs (DEPT); s=singlet; d=doublet; t= triplet; q=quartet.



Scheme 1. Epoxidation-Cyclization Products of Costunolide (1)



Scheme 2. Reduction of Santamarin (5)

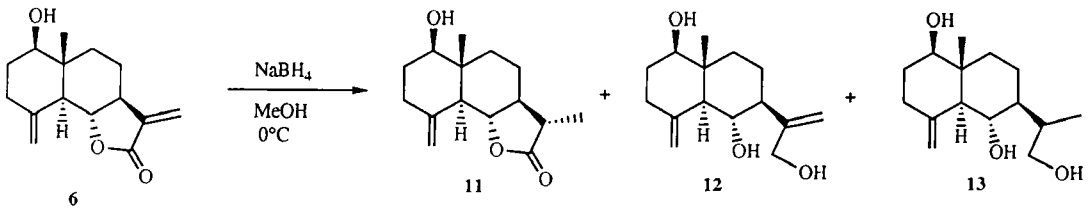
absorption was observed in the IR spectrum of **8**, a 1,4-epoxide structure was proposed. This was further confirmed by a prominent mass spectral molecular ion at m/z 248 , which was in agreement with the empirical formula $C_{15}H_{20}O_3$. Compound **8**, like the cyclization products **5**, **6** and **7**, must be formed through a carbocationic intermediate **A**, by intramolecular nucleophilic attack of the C-1 hydroxyl group at the cationic center C-4 giving the epoxy bridge (Scheme 1).

Reduction of santamarin (**5**) with $NaBH_4$ in MeOH at 0°C afforded dihydrosantamarin (**9**) [11] as the major and the triol **10** as a minor product (Scheme 2). The structure of dihydrosantamarin (**9**) was established by direct comparison with the spectroscopic data of an authentic sample and the ^1H NMR data reported in the literature [12]. Inspection of the ^1H-COSY spectrum of **10** revealed two geminally coupled proton doublets at δ 4.13 and 4.17 (J=12.6 Hz) which showed allylic couplings with the methylene signals at δ 5.10 and 5.24, indicating a reductive opening of lactonic ring of santamarin. The ^{13}C NMR data of **10** showed the presence of three oxygenated carbons with two methine signals at δ 76.0 and 71.2 and one methylene carbon absorption at δ 65.7. This suggested the presence of three hydroxyl groups which was further supported by a very strong IR absorption at 3361 cm^{-1}. The mass spectrum of **10** showed a prominent molecular ion at m/z 252 and the peaks at m/z 234 $[M-H_2O]^+$ and m/z 216 $[M-2H_2O]^+$ further supported its structure. The ^{13}C NMR spectrum exhibited four olefinic signals at δ 113.0, 121.8, 135.2 and 151.0 due to one methylene (C-13), one methine (C-3) and two quaternary carbons (C-4 and C-11), respectively. Complete assignments of the

^{13}C NMR signals were obtained by DEPT, 2D ^{1}H-COSY and inverse ^{1}H, ^{13}C-correlation experiments (Table 2).

Reduction of reynosin (**6**) using the same condition as described above for **5** provided, after VLC separation, the major product dihydroreynosin (**11**) and two minor lactonic ring opening products **12** and **13** (Scheme 3). Dihydroreynosin (**11**) was identified by spectral comparison with an authentic sample and data reported in the literature [6]. The ^{1}H NMR spectrum of **12** was similar to that of compound **10** as shown by inspection of its ^{1}H-COSY spectrum. Two geminally coupled proton doublets which appeared as an AB system at δ 4.11 and 4.16 (J=12.6 Hz) were allylically coupled to a pair of broad methylene singlets at δ 5.09 (H-13a) and 5.22 (H-13b), indicating that the lactone was reductively opened and the carbonyl group (C-12) in reynosin (**6**) was reduced to a primary alcohol moiety. The IR spectrum of **12** had a very strong absorption at 3364 cm^{-1} but no carbonyl absorption, which further supported the above argument. The mass spectrum of **12** showed a parent peak at m/z 252 and exhibited further prominent peaks at m/z 234 $[M-H_2O]^+$, 216 $[M-2H_2O]^+$ and 201 $[M-2H_2O-Me]^+$ which were in agreement with the proposed structure **12**. The ^{13}C NMR of **12** showed three oxygenated carbon signals at δ 78.8, 70.1 and 66.5 assigned to carbons 1,6 and 12. It also exhibited four olefinic signals with two methylene carbons at δ 108.3 (C-15) and 112.5 (C-13) and two quaternary carbons at δ 145.3 (C-4) and 151.3 (C-11). Combined application of ^{1}H-COSY, inverse ^{1}H,^{13}C-correlation and DEPT methods allowed assignments of all ^{13}C NMR peaks of **12** (Table 2).

The structure of compound **13** was unambiguously established by comparison of its spectroscopic data with those of **12**. Instead of the C-13-methylene signals at δ 5.09 and 5.22 in compound **12**, the ^{1}H NMR spectrum of **13** showed a methyl doublet at δ 0.93 coupled with a multiplet at δ2.07, indicating that it was a 11,13-dihydro derivative of **12**. This was further confirmed by its mass spectral data with a molecular ion at m/z 254, which was two mass units higher than the one observed for **12**. Other prominent peaks at m/z 236 $[M-H_2O]^+$, 218 $[M-2H_2O]^+$ and 203 $[M-2H_2O-Me]^+$ were also two mass units



Scheme 3. Reduction of Reynosin (**6**)

higher than the corresponding peaks of compound **12**. Its ^{13}C NMR spectrum showed three oxygenated carbon signals at δ 78.9 (C-1), 67.4 (C-6) and 66.8 (C-12) due to the three hydroxylated carbons. However, it only exhibited two olefinic signals; one was assigned to a CH_2 at δ 107.9 (C-15) and a quaternary carbon at δ 146.0 to C-4. This was in agreement with the fact of only one exocyclic double bond was present in the molecule. The ^{13}C NMR data of **13**, which were assigned on the basis of its ^1H-COSY and $^1H,^{13}C$-HETCOR analyses, are listed in Table 2.

EXPERIMENTAL

General. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AM 400 spectrometer. IR spectra were obtained on a Perkin-Elmer 1760X FT-IR spectrometer as a film on KBr plates. Mass spectra were recorded on a Hewlett-Packard 5971A GC-mass spectrometer. Vacuum liquid chromatographic separations were carried out on TLC grade silica gel (MN Kieselgel G).

Costunolide (**1**) and dehydrocostuslactone (**2**) were obtained from costus resin (*Saussurea lappa* Clark) obtained commercially as Costus Resinoid (Pierre Chauvet, S. A., France). About 11 g of costus oil was chromatographed by VLC [10] using hexane followed by mixtures of hexane and CH_2Cl_2 as solvent by increasing polarity, providing 18x200 ml fractions. The less polar fractions contained mainly dehydrocostuslactone (**2**) which upon recrystallization from hexane gave colorless crystals. More polar fractions provided, after recrystallization from hexane, costunolide (**1**) as colorless crystals.

Epoxidation-cyclization of costunolide (1). Costunolide (450 mg; 1.94 mmol) was dissolved in $CHCl_3$ (20 ml) and 220 mg of NaOAc and 446 mg of *m*-chloroperbenzoic acid (2.58 m mol) were added. The resulting solution was stirred for 1 hr., and then extracted with 10% aq. Na_2CO_3 (4x50 ml), washed with water (4x50 ml), dried over anhydrous Na_2SO_4. After removal of $CHCl_3$, 584 mg of a crude oil was obtained. VLC separation of the crude oil using hexane-EtOAc as solvent by increasing polarity provided

4 mg of **3**, 2 mg of **4**, 189 mg of **5**, 88 mg of **6**, 2 mg of **8**, 78 mg of a mixture of santamarin and magnolialide (**7**) and 50 mg of unreacted starting material (**1**).

1,4-Epoxy-11(13)-eudesmen-12,6-olide (**8**). $C_{15}H_{20}O_3$, colorless gum; IR cm^{-1}: 1772 (γ-lactone), 1464 (C=C), 1251, 1127 (CC(=O)OC), 1011, 968; EIMS (rel. int.): 248 $[M]^+$ (6.8), 233 $[M-Me]^+$ (24.7), 220 $[M-CO]^+$ (16.1), 215 (15.9), 204 (27.0), 190 (65), 175 (32.3), 163 (79.9), 145 (53.5), 135 (32.6), 123 (33.6), 119 (33.5), 107, 105 (45.6), 95 (53.6), 93, 91 (69.0), 79 (53.9), 67 (37.7), 53 (68.1), 43 (100); ^1H NMR data in Table 1 and ^{13}C NMR data in Table 2.

Reduction of santamarin (**6**). Santamarin (103 mg, 0.41 m mol) was dissolved in 25 ml CH_3OH, treated with $NaBH_4$ (250 mg, 6.6 m mol) at 0°C, stirred for 5 hr. The resulting solution was then acidified with 2N HCl to PH=1, diluted with water, extracted with CH_2Cl_2 (4x20 ml). After removal of solvent, about 87 mg of crude white powder was obtained. Further VLC separation of the crude material using hexane followed by mixtures of hexane and EtOAc as solvent by increasing polarity afforded 47 mg of dihydrosantamarine (**9**), 7 mg of the triol **10** and 20 mg of a mixture of **9** and **6**.

1β,6α,12-Trihydroxy-3,11(13)-eudesmadiene (**10**). $C_{15}H_{24}O_3$, colorless gum; IR cm^{-1}: 3361 (OH), 1652, 1440 (C=C), 1093, 1035; EIMS (rel. int.): 252 $[M]^+$ (5.2), 234 $[M-H_2O]^+$ (9.0), 216 $[234-H_2O]^+$ (9.9), 201 $[216-Me]^+$ (13.1), 183 $[201-H_2O]^+$ (12.1), 173 (11.8), 159 (16.1), 145 (24.7), 135 (28.3), 121 (52.3), 107 (100), 97 (96.6), 95, 93, 91 (52.7), 81, 79 (50.2), 77, 69, 67 (37.4), 55 (52.8), 43 (55.5), 41 (61.1); ^1H NMR data in Table 1 and ^{13}C NMR data in Table 2.

Reduction of reynosin (**7**). Using similar condition as above, reynosin (75.5 mg, 0.30 m mol) was dissolved in 10 ml CH_3OH, treated with $NaBH_4$ (240 mg, 6.3 m mol) at 0°C, stirred for 2 hr. The reaction solution was neutralized with 2N HCl and diluted with water. The resulting mixture was extracted with CH_2Cl_2 (4x20 ml) and evaporated in vacuum providing 70 mg of crude material. VLC separation of the crude using hexane-EtOAc as solvent by increasing polarity afforded 46 mg of dihydroreynosin (**11**), 12 mg of triol **12** and 9 mg of **13**.

1β,6α,12-Trihydroxy-4(15), 11(13)-eudesmadiene (**12**). $C_{15}H_{24}O_3$, colorless crystals; IR cm^{-1}: 3364 (OH), 1653, 1457 (C=C), 1007, 867; EIMS (rel. int.): 252 $[M]^+$ (3.4), 234 $[M-H_2O]^+$ (23.2), 219 $[234-Me]^+$ (6.1), 216 $[234-H_2O]^+$ (10.4), 201 $[216-Me]^+$ (18.7), 187, 185 (30.9), 173 (18.2), 159 (27.3), 145 (39.8), 135 (35.1), 133, 131, 121 (63.6), 109, 107 (100), 105, 95, 93 (72.6), 91 (70.5), 81, 79 (83.0), 67 (52.3), 55 (63.6), 43 (55.6), 41 (62.8); ^1H NMR data in Table 1 and ^{13}C NMR data in Table 2.

1β,6α,12-Trihydroxy-4(15)-eudesmene (**13**). $C_{15}H_{26}O_3$, colorless gum; IR cm^{-1}: 3381 (OH), 1655, 1455 (C=C), 1005, 731; EIMS (rel. int.): 254 $[M]^+$ (0.2), 236 $[M-H_2O]^+$ (10.2), 221 $[236-Me]^+$ (5.0), 218 $[236-H_2O]^+$ (14.3), 203 $[218-Me]^+$ (16.1), 187 (26.9), 177 (14.3), 159 (58.1), 145 (28.1), 134 (80.0), 121 (100), 109, 107 (83.9), 93 (56.2), 91, 81 (97.9), 69 (33.3), 67, 55 (49.0), 43 (40.1), 41 (39.1); ^1H NMR data in Table 1 and ^{13}C NMR data in Table 2.

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REFERENCES

1. Fischer, N. H., In *Biochemistry of the Mevalonic Acid Pathway to Terpenoids*; (Towers, G. H. N., Stafford, H. A., eds.). pp 161-201. Plenum Press, New York, 1990.
2. Parodi, F. J., F. R. Fronczek and N. H. Fischer, *J. Nat. Prod.*, **1989**, *52*, 554.
3. Castañeda-Acosta, J., N. H.Fischer and D. Vargas, *J. Nat. Prod.* **1993**, *56* , 90.
4. Picman, A. K., *Biochem. Syst. Ecol.*, **1986**, *14*, 255.
5. Fischer, N. H., In *Plant terpenoids as allelopathic agents*; (Harborne, J. B., Tomas-Barberan, F. A., eds.). pp 377-398. Oxford University Press, New York, 1991.
6. Ogura, M., G. A. Cordell and N. R.Farnsworth, *Phytochemistry* , **1978**, *17*, 957.
7. Rodrigues, A. A. S., M. Garcia and J. A. Rabi, *Phytochemistry* , **1978**, *17*, 953.

8. Pathak, S. P., B. V.Bapat and G. H. Kulkarni, *Indian J. Chem.*, **1970**, *8*, 471.

9. El-Feraly, F. S., Y. M. Chan and D. A. Benigni, *Phytochemistry* , **1979**, *18*, 881.

10. Coll, J. C. and B. T. Bowden, *J. Nat. Prod.*, **1986**, *49*, 934.

11. El-Feraly, F. S. and D. A. Benigni, *J. Chem. Soc. Perkin Trans. I*, **1983**, 355.

12. Clark, A. M. and C. D. Hufford, *J. Chem. Soc. Perkin Trans. I*, **1979**, 3022.

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