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A new sesquiterpene lactone from the roots of *Saussurea lappa*: Structure–anticancer activity study

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ABSTRACT

The dried roots of *Saussurea lappa*, called costus roots, are used in the traditional system of medicine for the treatment of cancer. In our investigation for the anticancer constituents from the hexane extract of this plant, a new sesquiterpene (**1**) was isolated along with the known compounds costunolide (**2**), β-cyclocostunolide (**3**), dihydro costunolide (**4**) and dehydro costuslactone (**5**). Their structures were established by the extensive spectroscopic analyses. In addition, costunolide and β-cyclocostunolide derivatives were synthesized using Michael-type addition reaction of NaOMe to the α-methylene-γ-lactone moiety. All the compounds were tested for their in vitro cytotoxic activity. Compound **1** exhibited potent cytotoxic activity and other compounds displayed moderate activity.

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Saussurea lappa Clarke (Compositae) is indigenous to India and Pakistan, where it grows in the Himalayas at 2500–3500 m elevations. Sesquiterpene lactones are the most common constituents of *S. lappa*.[1] Dried 4- to 5-year-old roots of this plant are known as costus root and have reputation for their usage in traditional systems of India, China and Japan.[2] It has been reported that sesquiterpenes exert their antitumour activity by triggering apoptosis in human leukaemia cells. A thorough literature survey indicated that sesquiterpene lactones are rich in costus roots (3% of fresh weight), of which (+)-costunolide is the chief constituent,[3] which has shown potent anticancer activity and has been considered as a potential candidate for various types of tumours.[4,5]

As part of our continuing efforts directed towards the discovery of the structurally interesting and biologically active compounds from the Indian medicinal plants,[6] we have examined the chemical constituents of a sample of *S. lappa* procured from local market. These efforts resulted in the identification of new compound (**1**) including several known compounds costunolide (**2**), β-cyclocostunolide (**3**), dihydro costunolide (**4**) and dehydro costuslactone (**5**). The present study focuses on the structural elucidation of the new sesquiterpene lactone (**1**) and investigation of the structure–anticancer activity relationships for costunolide and β-cyclocostunolide derivatives.

The shade-dried costus roots (1 kg) were extracted with hexane using Soxhlet apparatus to give 25 g of extract, which was subjected to column chromatography over silica gel (100–200 mesh) and eluted with hexane/ethyl acetate in the increasing order of polarities to afford three fractions. Fraction I was further subjected to column chromatography and eluted with 99:1 (hexane/ethyl acetate) to give compounds **1**, **2** and **3**. Similarly, the other fractions were also rechromatographed on silica gel (100–200 mesh) column and eluted with 2% EtoAc in hexane to yield the compounds **4** and **5**.

Compound **1** was obtained as a white solid of mp: 76–78 °C with optical rotation $[\alpha]_D$ +152.2 (*c* 1.0, $CHCl_3$). The molecular formula $C_{15}H_{22}O_2$ has been determined from the molecular ion peak at *m/z* 235 (M^++H) in the positive ion ESIMS and by the high resolution MS measurement. The IR spectrum of **1** showed the absorption bands at 1751 cm^{-1} indicating the presence of γ-lactone functionality. The ^1H NMR ($CDCl_3$) spectrum of **1** has all the features of a costus lactone. It has an α-*exo*cyclic methylene group with one proton appearing at δ 5.6 (d, J = 2 Hz) and another at δ 6.2 (d, J = 2 Hz). It has also showed the signals assignable to two methyls, one appearing as a doublet at δ 1.1 (d, J = 8 Hz) and another appearing as a singlet at δ 1.2. It further has an olefin proton at δ 5.15 (d, J = 4.2 Hz). The proton on the carbon atom, where the lactone ring is fused to the ten-membered cyclic ring appearing at δ 4.81 signifies the bridge proton (H-6) and the other bridge proton

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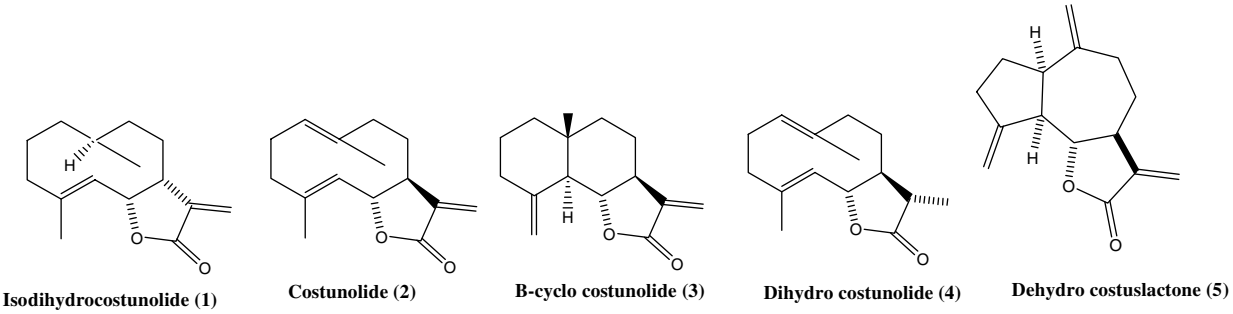


Figure 1. Structures of the compounds **1**–**5**.

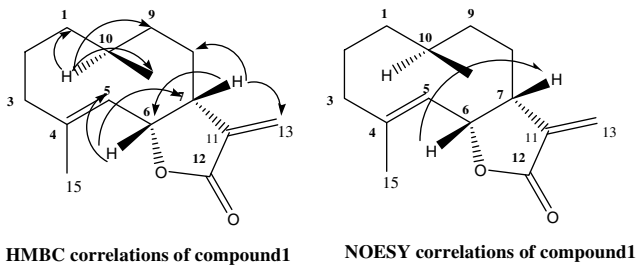


Figure 2. 2D NMR connectivities of compound **1**.

resonated as a multiplet at δ 3.6 (H-7). The ^{13}C NMR spectrum of the compound **1** in $CDCl_3$ exhibited 15 carbons; DEPT experiment confirmed the presence of two methyls, six methylenes, four methines, and three quaternary carbons. Finally, all the ^{1}H NMR and ^{13}C NMR resonances were assigned by applying homo- and hetero-nuclear NMR methodologies.[7] The important 2D NMR (HMBC and NOESY) connectivity of the compound **1** are depicted in Figure 2. Based on these data, the structure of compound **1** was identified as a new costus lactone ({3aR,9S,11aS,E)-5,9-dimethyl-1-methylene-3,3a,7,8,9,10,11,11a-octahydro-1H-cyclopenta[10]annulen-2(6H)-one}) and named as isodihydrocostunolide (Fig. 1).

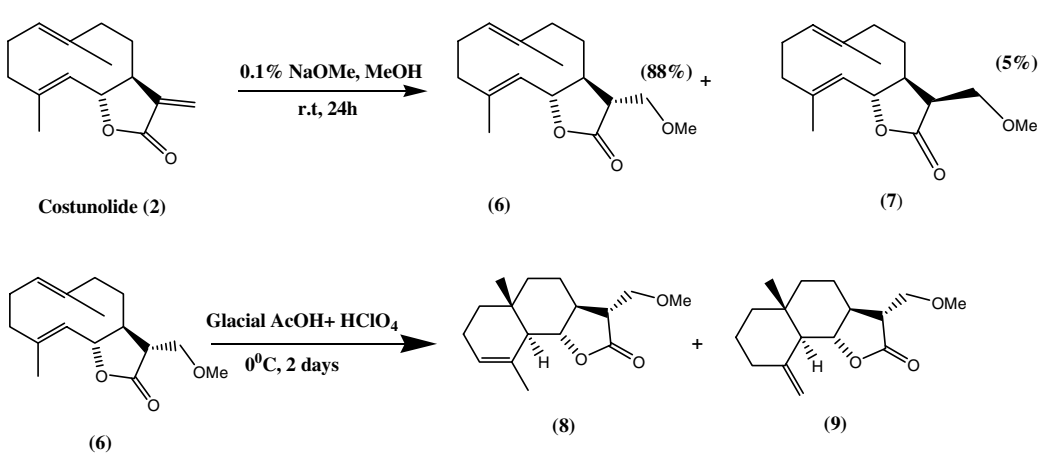
The relative stereochemistry of compound **1** was deduced from its NOESY spectrum and also by comparing the ^{1}H NMR and ^{13}C NMR data with those reported in the literature.[8] The ^{1}H NMR values of H-6, H-7 and C-6 and C-7 are indicative of a *cis*-fused system as in 6, 12-edesmanolides.[8] This was further confirmed by the NOE correlation between H-6 and H-7 protons. The carbon resonances of the C-6 and C-7 in the ^{13}C NMR at δ 76.41 and δ 37.64 are indicative of *cis*-fused system with bridge hydrogens positioned in β-orientation as shown in Figure 1. Further, the absence of correlation between H-6, H-10 and H-7, H-10 signals clarified the stereochemistry of the H-10 to be in α-configuration. On the basis of this evidence, the relative stereochemistry was determined as shown in Figure 1.

Compounds **2**–**5** were identified as costunolide, β-cyclocostunolide, dihydro costunolide and dehydrocostuslactone, respectively, on the basis of their physicochemical properties and NMR spectral data, which were in good agreement with those published previously.[9]

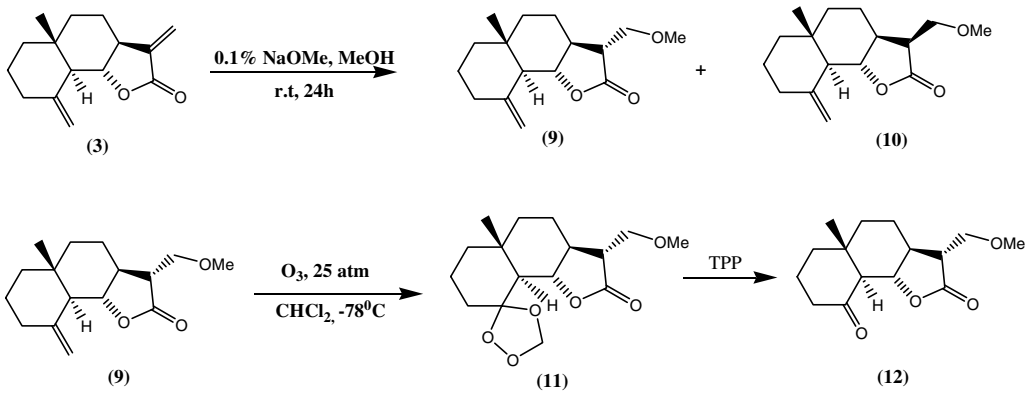
Chemical modification of sesquiterpenes: In an attempt to deepen the understanding of the structure–anticancer activity relationships, several chemical reactions were performed parallely starting with costunolide and β-cyclocostunolide.[10] As shown in Scheme 1, Michael-type addition of the α-methylene-γ-lactone of costunolide with NaOMe in methanol afforded the compounds **6** and **7**. Further, compound **6** was treated with a mixture of glacial acetic acid and perchloric acid mixture to yield **8** and **9** in a ratio of 1:1.[10]

Similarly, β-cyclocostunolide (**3**) was also treated with 0.1% NaOMe in methanol to yield the compounds **9** and **10** and further ozonolysis of compound **9** afforded compound **11**, which was treated with TPP to yield compound **12**.[11] Based on the literature, the stereochemistry at position −11 in the synthesized derivatives (**6**–**12**) has been assumed (Scheme 2).

In vitro cytotoxicity evolution: All the isolates and its derivatives were tested for in vitro cytotoxicity on cancer cell lines and IC_{50} values were calculated in microgram (μg/mL).[12] The cell lines used for this study are the colon cancer (colo-205), skin cancer (A-431), breast cancer (MCF-7) and lung cancer (A-549). The cytotoxicity data are summarized in Table 1. The structural differences of the



Scheme 1. Synthesis of costunolide derivatives.



Scheme 2. Synthesis of β-cyclocostunolide derivatives.

Table 1
Cytotoxicity of the compounds

Compound	Cell lines (IC_{50}, μg/mL)			
	Colo-205	A-431	MCF-7	A-549
1	27.03 ± 0.67	107 ± 7.46	35.05 ± 9.37	125 ± 0.95
4	134 ± 7.6	NA	57.94 ± 4.71	NA
6	220 ± 59.13.0	NA	75.05 ± 9.32	NA
9	109.81 ± 10.6	NA	88.49 ± 17.1	NA
11	NA	NA	180.29 ± 33.8	NA
12	NA	NA	103.38 ± 19.75	NA

NA, not active.

sesquiterpene lactones significantly affected the anticancer activity.

It has been reported that *exo*-methylene group on lactone part of the sesquiterpenes is required for eliciting cytotoxicity.[5] The present study also revealed the importance of *exo*-methylene group on the sesquiterpenes for their cytotoxicity. As evident from Table 1, compound **1** exhibited good activity on Colo 205, A431 and MCF-7 cell lines and moderate activity on A549 cell lines. However, when the position 13 (*exo*-double bond) was substituted with methoxy group (**6**, **9**, **11** and **12**), cytotoxicity decreased drastically against all the tested cell lines. In the similar fashion, when C-4 double bond in the β-cyclocostunolide (**9**) was converted into carbonyl (**12**), the activity was lost in all cell lines except for MCF-7. It is interesting to note that all the tested compounds were inactive on A-549 cell line except for compound **1**. Hence, the compound **1** was found to possess anticancer activity towards all cell lines.

In summary, *S. lappa*, of the Compositae family has been used as a traditional medicine for the treatment of cancer. The compounds responsible for this activity are yet to be determined. In this study, we focused to investigate anticancer principles from the *S. lappa* and found a new sesquiterpene lactone with good activity on the tested cell lines. In addition, we also studied costunolide and related derivatives for the anticancer activities and they displayed moderate activity (IC_{50}, 27.03–180 μg/mL).

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7. NMR data of compound 1: ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.2 (1H, d, *J* = 2 Hz, H-13a), 5.65 (1H, d, *J* = 2 Hz, H-13b), 5.15 (1H, d, *J* = 6 Hz, H-5), 4.8 (1H, m, H-6), 3.6 (1H, m, H-7), 2.10 (1H, dd, *J* = 2.7 Hz, H-3a), 2.5 (1H, m, H-10), 1.9–1.7 (1H, m, H-3b), 1.68–1.40 (8H, m, H-1, 2, 9, 8), 1.2 (3H, s, 15-Me), 1.1 (3H, d, *J* = 8 Hz, 14-Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.73 (C-1), 32.63 (C-2), 42.64 (C-3), 148.92 (C- 4), 118.54 (C-5), 76.41 (C-6), 39.46 (C-7), 32.68 (C-9), 41.70 (C-8), 37.54 (C-10), 139.87 (C-11), 170.36 (C-12), 121.54 (C-13), 22.54 (C-14), 28.56 (C-15).
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12. http://www.atcc.org/pdf/30-1010 k.pdf. **In vitro cytotoxicity evaluation**: All the isolates and its derivatives were tested for in vitro cytotoxicity on different cancer cell lines by MTT assay. The cell lines used in this study were colo-205 (colo-cancer), A-431 (skin cancer), MCF-7 (breast cancer) and A-549 (lung cancer). All the cells were obtained from National Center for cellular Sciences (NCCS), Pune, India. DMEM (Dulbecco's modified Eagles medium), MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide], trypsin, EDTA were purchased from sigma chemicals Co. (St. Louis, MO) and Fetal bovine serum were purchased from Gibco. Cells in 96-well plate were incubated with compounds tested for 48 h at 37 °C in DMEM with 10% FBS medium. Then, the above media was replaced with 90 μl of fresh serum-free DMEM and 10 μl of MTT reagent (5 mg/mL) and plates were incubated at 37 °C for 4 h, thereafter above media was replaced with 200 μl of DMSO and incubated at 37 °C for 15 min. The absorbance at 570 nm was measured on a spectrophotometer (Spectra max, Molecular devices). The values for each point were calculated from the triplicate wells. IC_{50} values were calculated in μg/mL from plot (% inhibition vs concentration).