ISSN:0975-5772

Robinson A *et al* / Journal of Pharmaceutical Science and Technology Vol. 2 (4), 2010, 207-212

Isolation of α-amyrin eicosanoate, a triterpenoid from the roots of *Saussurea lappa* Clarke - Differential solubility as an aid

Robinson A[1], Sunkara Yashvanth[1], Suresh Babu K[1] Roa J M[1] and Madhavendra S S[2*]

[1]Natural Product Chemistry Division, Indian Institute of Chemical technology, Hyderabad-500007, AP., India

[2]Electron Microscopy Center, Indian Institute of Chemical technology, Hyderabad-500007, AP., India

Abstract:

The plant *Saussurea lappa* is well known for its varied medicinal properties. Alpha-amyrin eicosanoate (1), a triterpenoid and the sesqueterpene lactones, Dehydrocostus lactone (2), Beta-cyclo costunolide (3), costunolide (4), Epoxyisozaluzanin C 4α 15-epoxide (5) and Epoxyisozaluzanin C 11α 13-epoxide (6) were isolated from the chloroform extract of the roots of *Saussurea lappa.* Column Chromatography, HPLC, HPTLC-UV, LC-MS, MS, IR, SEM, EDAX and NMR techniques were employed for the isolation and characterization. Differential solubility of the compound 1 in methanol facilitated its easy isolation from the rest. Alpha-amyrin eicosanoate is reported for the first time from the roots of *Saussurea lappa.*

Keywords: *Saussurea lappa*, Alpha-amyrin eicosanoate, sesqueterpene lactones Costus roots

Introduction:

Saussurea lappa (Compositae) is cultivated as a medicinal plant in the Himalayas. Most of the roots are exported to China and Japan and the plant forms quite a large article of commerce in Kashmir, the trade being controlled by the State. It has been placed on the CITES I list of endangered species (Kuniyal et al., 2005). It is known as Costus (Kirtikar and Basu, 2001). *Costi amari radix* or costus root was an important item of Roman trade with India, and is believed to have been the dried root of *Saussurea lappa* (Moeslinger et al., 2000). Pandey et al., 2007 have reviewed extensively the medicinal importance of *Saussurea lappa*. The plant is rich in sesquiterpenenoid lactones and terpenoids. Several reports are available on their isolation (Robinson et al., 2008; Kim et al., 2008; Rao et al., 1960; Govindan & Bhattacharya, 1977; Lalla et al., 2004, 2005; Talwar et al., 1992; Kalsi et al., 1983). However, the quantities of the individual compounds are very low (Ghosh et al., 1929) and the task of their isolation is cumbersome. Extensive work was carried out on aqueous, alcoholic and hexane extracts. Much is lacking on chloroform extract. Hence, an attempt was made in order to explore new chemicals in the chloroform extract of the roots of *Saussurea lappa*.

Of the six compounds reported here in, Compound 1 is reported for the first time in the roots of *Sassurea lappa*. However, it was reported earlier from the aerial parts of the plant *Messerschmidia sibrica* (Boraginaceae) by Song et al., 1996. Compounds 2-6 are reported earlier from *Sassurea lappa* but their isolation protocols are very much different from that of the current study.

Materials and Methods:

Plant Material, Extraction and Fractionation

Commercial sample of *Sassurea lappa* roots was obtained from Kishan Lal Dawasaz (Ayurveda & Unani), Ladd Bazar, Hyderabad. Powdered root sample was cold extracted in chloroform for a week (Maceration). The extract was filtered, concentrated and checked for the presence of compounds by running TLC. TLC showed distinct spots. The extract was further evaporated to dryness. The concentrated extract (residue) was subjected to column chromatography over silica gel (60-120mesh) The column was eluted with solvents of increasing polarity (EtOAc in Hexane; 0.5-3.0%). Resolution of the components was monitored by TLC and similar types of fractions were combined. Total 28 fractions were collected, first thirteen fractions obtained with 0.5% EtOAc in hexane were showing freak spots in TLC. They are pooled and named as Fraction-I. Remaining 15 fractions obtained with 1-3% EtOAc in hexane were pooled and

designated as Fraction –II. Fraction-II showed a short UV active single spot (Rocket shaped) in TLC. The spot turned pink on developing with 10% methanol in H_2SO_4.

Precipitation with Methanol

Fraction -II (2mg) was stirred with 1 ml of methanol. This resulted a white crystalline material and the same is designated as compound-1. The methanol was filtered and designated as Fraction-IIa. Compound-1 was subjected to IR (Thermonikolet, Nexus 670), UV(Rayleigh 2100) SEM(Hitachi S3000N), EDAX(Oxford, Link-ISIS 300), ESI-Mass(Micromass, Autospec) and NMR(Bruker 400MHz) analysis.

Separation of Sesquiterpene lactones by Liquid Chromatography

Fraction-IIa was subjected to Liquid Chromatography (Agilent technologies, 1100 series) with 1:1 ACN and water. Fraction-IIa resulted three prominent peaks at 7.881, 8.546 and 8.925 minutes. A rudimentary peak was also noticed at 9.915 minutes. The prominent fractions are subjected to LC-MS and designated as Compound-2, Compound-3 and Compound-4 respectively.

Separation of Sesquiterpene lactones by HPTLC

Fraction-II (2mg) was dissolved in 5 ml methanol and subjected to HPTLC(Camag, Linomat) with 6:2:2 toluene: Acetone: Methanol as a mobile phase. This resulted into 4 spots with Rf values, 0.43, 0.52, 0.60, and 0.86. UV scans of first and last spots match with that of the compounds (2,3,4), 1 respectively. The middle spots are eluted with chloroform and further characterised in IR & ES-Mass and designated as compound 5 and 6.

Results and Discussion:

HPTLC of fraction II showed 4 distinct spots (Fig. 1a) with Rf. Values 0.43, 0.52, 0.60 and 0.86. The λ_{max} of the same are 220, 287, 289 and 243nm respectively (Fig.1b). λ_{max} of the Compound 1 crystallised directly from Fraction I is matching with that of peak 4. Literature values (λ_{max}) of compounds 2, 3 and 4 (Vijayakannan et al., 2006) are matching with that of the λ_{max} of peak 1. The peak areas of the spots are 3238, 7318, 5399 and 19334 and the Percent peak areas are 9.18, 20.74, 15.30 and 54.79 respectively. Hence, the concentration of Compound 1 > Compound 5 > Compound 6> (Compounds 2+3+4) in Fraction II.

Compound 1 was precipitated as white granular crystals (Fig.2a depicts the SEM picture) from Fraction II by restricting the methanol quantity used. When excess methanol was used it was getting dissolved. Differential solubility of the compound has facilitated its easy isolation. EDAX analysis indicated the presence of C and O only, which is an indication that the compound was not an alkaloid or any N, S containing compound. It gave a positive coloration with Libermann-Burchard reagent for triterpenoid. ESI MS (Fig. 2b) exhibited the Na-adduct peak at *m/z* 743 (M+23). IR spectrum showed absorptions at 1764.73 (ester carbonyl) and 1641.83 cm^{-1} (double bond). ^{1}HNMR ($CDCl_3$): δ 0.69, 0.70, 0.71, 0.73, 075, 0.8, 0.85, 1.1, 1.2 (m, 9xMe), 1.2(br, s, - $(CH_2)_{17}$ -), 4.5 (dd, H-3), 5.15(t, H-12). All these characteristics of compound are in agreement with the reported values (Song et al., 1996) and the compound is identified as α-amyrin eicosanoate (Fig. 3a). Amyrin-octadecanoate, (Chapman & Hall Data Base No. KDM59-E, CAS Registry Number: 63195-78-8), eicosanoate, α - amyrin-stearate, and amyrin were already reported in Sassurea *lappa* However, the present report on the presence of α-amyrin eicosanoate in the roots of Saussurea lappa is novel.

Mass values (M+1) of the three prominent peaks (Fig. 4a) in Liquid chromatographic separation of Fraction IIa are 231, 233 and 233 respectively (Fig. 4b). Retention times,

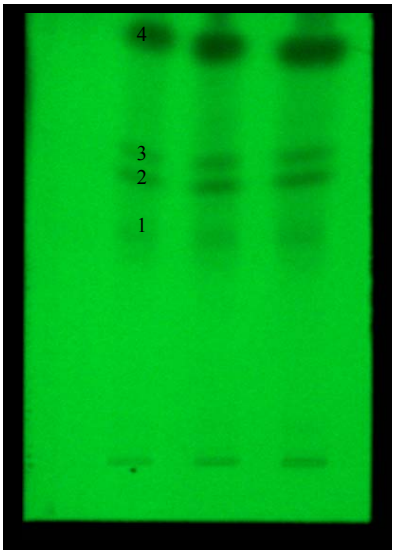

Figure 1a: HPTLC Plate of Fraction II

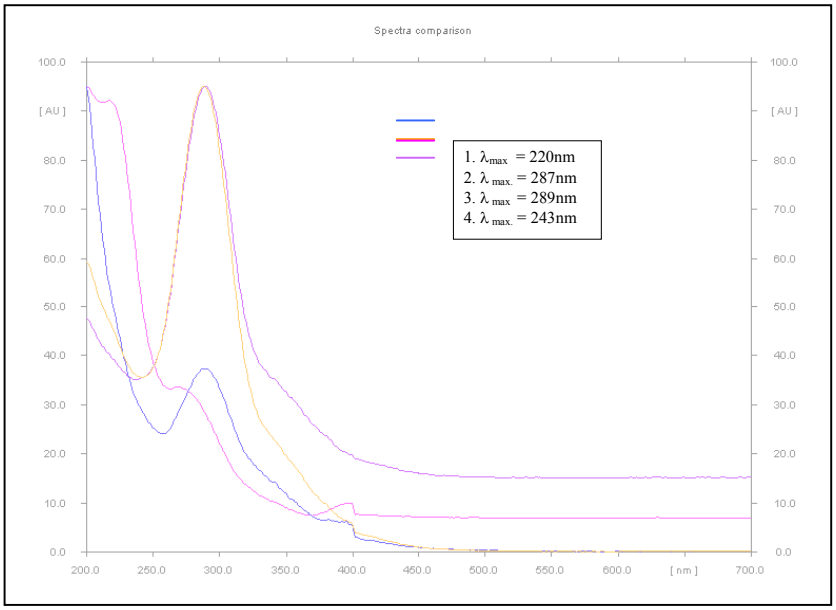

Figure 1b: UV scans of HPTLC

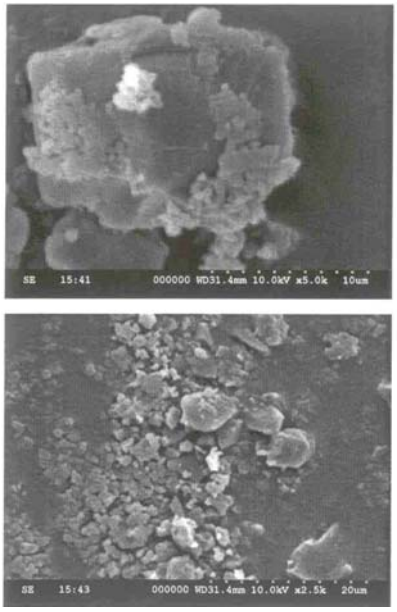

Figure 2a: SEM Pictures of Compound 1 enlarged granule like crystal at the top

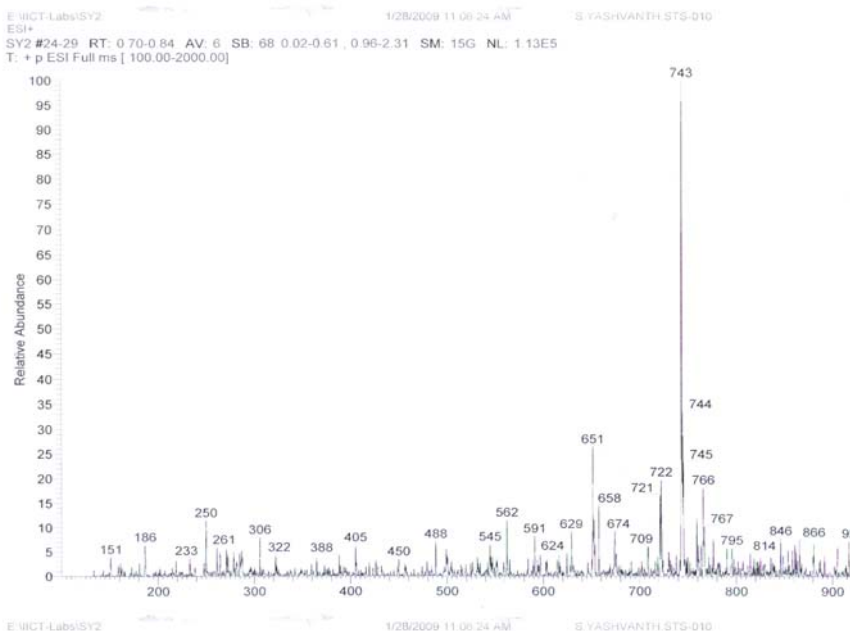

Figure 2b: Mass Spectrum of Compound 1

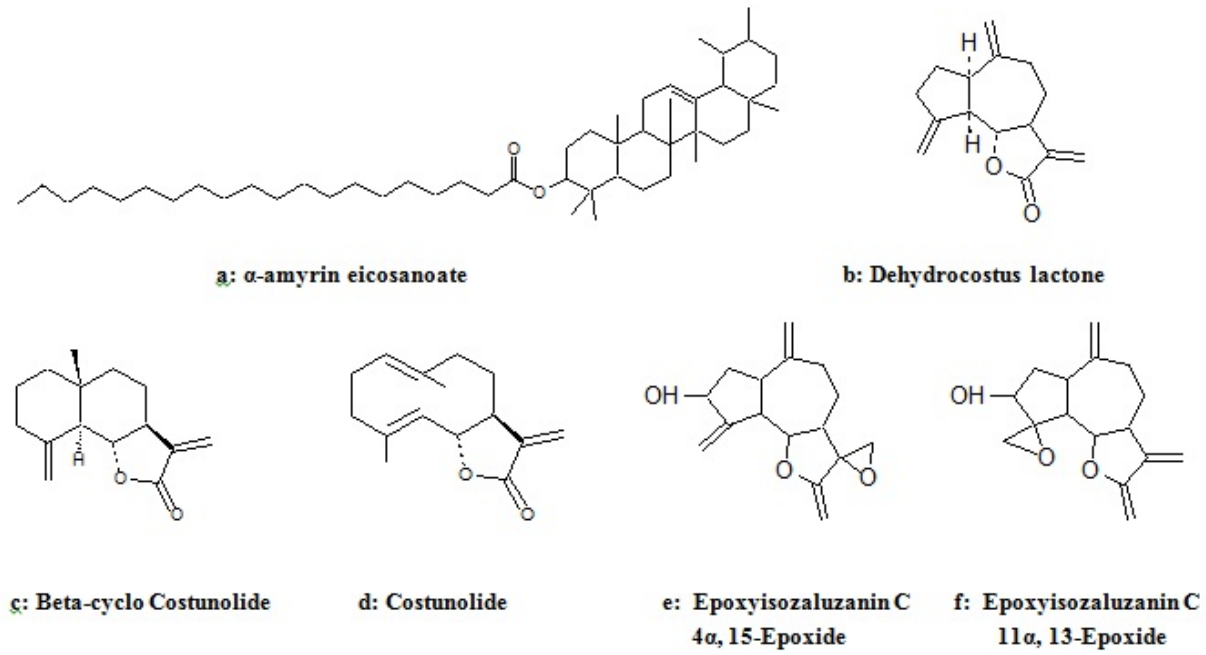

Figure 3: Structures of the molecules isolated

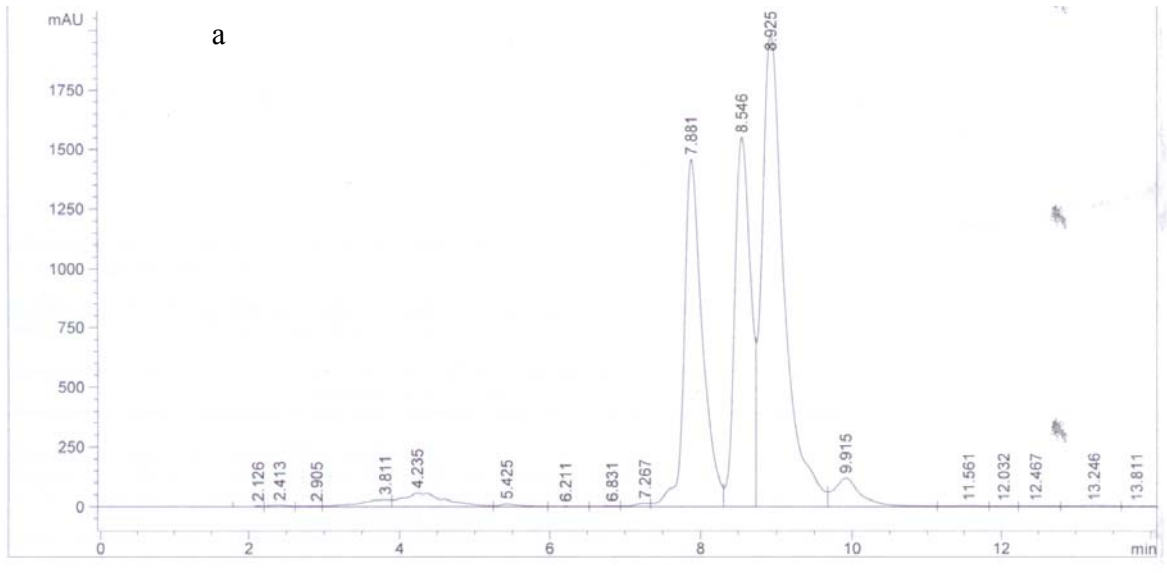

Figure 4: a. LC Spectrum

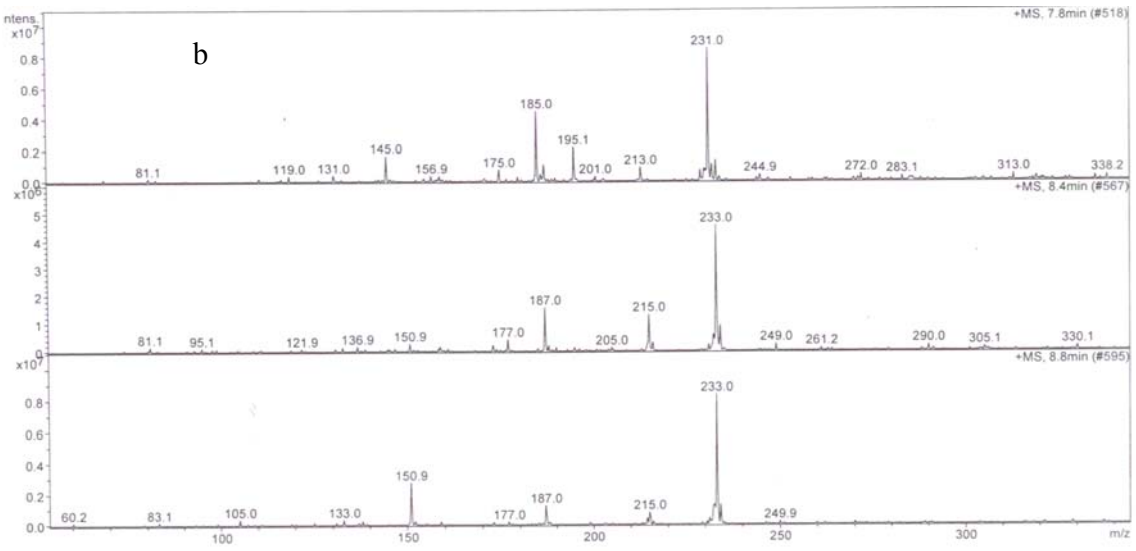

Figure 4: b. MS. Spectrum of Fraction IIa

Mass values and IR band for γ-lactone functionality (1763.7 cm^{-1}) of the same are very much identical with the reported values (Robinson et al. 1998). They have isolated the compounds corresponding to the peaks through preparative chromatography. The compounds are Dehydrocostus lactone, Beta-cyclo costunolide and Costunolide respectively (Fig. 3b, c and d respectively).

Compounds 5 and 6 eluted from the HPTLC plates showed same Mass value - (M+1) 279. Compound 5 exhibited IR V_{max} cm^{-1} – 2925.49, C-H stretching vibration of Epoxy ring; 1635.14, C=O stretching and 764.58, C-C bond stretching of C-O bond in epoxy ring. This data matches with that of poxyisozaluzanin C 4α, 15-Epoxide (Fig.3e), a known compound reported from *Sausssurea lappa* (Chapman & Hall Data Base No. HJN91-W, CAS Registry No. 153482-0807). Compound 6 showed IR V_{max} cm^{-1} 1630.46, C=O stretching and 764.33, C-C bond stretching of C-O bond in epoxy ring. This data matches with that of Epoxyisozaluzanin C 11α, 13-Epoxide (Fig.3f), a known compound reported from *Sausssurea lappa* (Chapman & Hall Data Base No. OGW48L, CAS Registry No. 205492-13-3).

Acknowledgements:

The authors thank Dr. J. S. Yadav, Director, IICT for constant encouragement. Thanks are also due to Dr. P. Sitadevi for HPTLC-UV work.

References:

[1] C. P. Kuniyal, Y. S. Rawat, S. S. Oinam, J. C. Kuniyal and S. C. R. Vishwakarma. Kuth (*Saussura lappa*) cultivation in the cold desert environment of the Lahaul valley, northwestern Himalaya. India: arising threats and need to revive socio-economicvalue. Biodiversity and Conservation 14: 1035-1045 (2005).

[2] K. R.Kirtikar and B. D. Basu. Indian Medicinal Plants Vol. 6. Second edn. Oriental Enterprises, 2001, pp. 1961-1965.

[3] T. Moeslinger, R. Friedl, I. Volf, M. Brunner, E. Koller and P. G. Spieckermann. Inhibition of inducible nitric oxide synthesis by the herbal preparation Padma 28 in macrophage cell line**.** Can J. Physiol Pharmacol. 78(11): 861-866 (2000).

[4] M. Pandey, M. S. Rastogi and A. K. S. Rawat. Saussurea costus: Botanical, Chemical and pharmacological review of an ayurvedic medicinal plant. J. Ethnopharmacology 110: 379-390 (2007).

[5] A. Robinson, T. Vijaya Kumar, E. Sreedhar, V. G. M. Naidu, S. Rama Krishna, K. Suresh Babu, P. V. Srinivas and J. Madhusudhana Rao. A new sesquiterpene lactone from the roots of Sassurea lappa: Structure-anticancer study.

Bioorganic & Medicinal Chemistry Letters. 18: 4015-4017 (2008).

[6] S. H. Kim, M. Danilenko and T. S. Kim. Differential enhancement of leukemia cell differentiation without elevation of intracellular calcium by plant-derived sesquiterpene lactone compounds. Br. J. Pharmacol. 155: 814-825 (2008).

[7] S. A. Rao, G. R. Kelkar, and S. C. Bhattacharya. The structure of costunolide, a new sesquiterpene lactone from costus root oil. Tetrahedron, 9: 275-283 (1960).

[8] S. V. Govindan, and S. C. Bhattacharyya. Alantolides & cyclocostunolides from *Sassurea lappa* Clarke (Costus Root). Indian J. Chem. 15B: 956-957 (1977).

[9] J. K. Lalla, P. D. Hemarpurkar and S. A. Mukherjee. Estimation of the Saussurine content of root powder and a Tablet formulation, prepared from *Saussurea lappa.* J. Planar Chromatography, 17(1): 36-39 (2004).

[10] J. K. Lalla, P. D. Hemarpurkar and S. A. Mukherjee. Isolation and characterization of Saussurine. Indian Drugs 42(4): 209-212 (2005).

[11] K. K.Talwar, I. P. Singh and A. S. Kalsi. A sesquiterpenoid with plant growth regulatory action from *Saussurea lappa*. Phytochemistry 31(1): 336-338 (1992).

[12] P. S. Kalsi, S. Sharma, and G. Kaur. Isodehydrocostus lactone and isozaluzanin C, Two guaianolides from Sassurea lappa. Phytochemistry, 22: (9), 1993-1995 (1983).

[13] S. N. Gosh, R. Chattergee and A. Dutta. 1929. Chemical examination of the roots and leaves of Saussurea lappa, Clarke. Part I. J. Indian Chem. Soc. 6: 517-522 (1929).

[14] Z. Z. Song, B. G. Wang and Z. J. Jia. Lignan derivatives of caffeic acids and terpenoids from Messerschmidia sibirica L. ssp. Indian J. of Chemistry. 35B: 955-959 (1996).

[15] R. Vijayakannan, M. Karan, S. Dutt, V. Jain and K. Vasist. A rapid densitometric TLC method for simultaneous Analysis of costunolide and dehydrocostus lactone in *Saussurea costus*. Chromatographia 63(5/6): 277-281 (2006).