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VASOACTIVE SUBSTANCES FROM *SAUSSUREA LAPPA*

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In our search for pharmacologically active compounds from crude drugs of plant-origin, we found that a MeOH extract of *Saussurea lappa* Clarke (Compositae) inhibited contractions of the rabbit-isolated aorta induced by KCl at concentrations not affecting those induced by norepinephrine. The present paper deals with the isolation and characterization of these active substances from the roots of *S. lappa*.

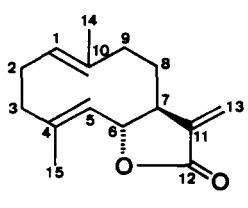
EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotation was recorded on a Jasco DIP-180 digital polarimeter. Ir spectra were obtained with a Shimadzu IR-27G ir spectrophotometer. Mass spectra were obtained by using a Shimadzu LKB 9000B gas chromatograph-mass spectrometer. ^{1}H-nmr spectra were recorded on a JOEL JNM-GX-400 FT NMR spectrometer. Silica gel 60 F_{254} (0.25 mm) and silica gel 60, Merck, were used for tlc and for column chromatography, respectively.

PLANT MATERIAL.—Roots of *S. lappa* were purchased from Nippon Hunmatsu Yakuhin, Ltd., Osaka, Japan, and a voucher specimen is on deposit in our institute.

BIOASSAY.—Rabbits (2-3 kg) were sacrificed by cervical dislocation. The procedure for preparing the aorta and technique for measurement of the mechanical response was carried out as described previously (1).

ISOLATION OF VASOACTIVE SUBSTANCES.—The dried roots (1 kg) were extracted three times with cold MeOH, and the combined extract was evaporated in vacuo. The residue was partitioned between EtOAc and H_2O. The aqueous layer was then extracted with *n*-BuOH. The bioactivity was found to be concentrated in the EtOAc extract, the residue (113 g) of which was chromatographed on a silica gel (1800 g) column, eluted with C_6H_6/EtOAc mixtures of increasing polarity, and fractionated successively. After monitoring using tlc, the fractions were combined and subjected to the bioassay. The active fractions were again combined and evaporated to dryness in vacuo. A 10-g portion of the residue (35.8 g) was chromatographed on a silica gel (300 g) column using *n*-hexane-Me_2CO (97:3) to yield active Compound **1** (3.3 g), followed by active Compound **2** (3.9 g). Compound **1**, $C_{15}H_{20}O_2$, colorless needles, mp 108-109° (recrystallized from *n*-hexane/Me_2CO) [lit. mp 106-107° (2)]; $[\alpha]_D$ +111° (*c* 0.11, $CHCl_3$) [lit. +125° (*c* 0.6, $CHCl_3$) (2)]; ir ν max (KBr) 1770, 1670, 1440 cm^{-1}; eims (rel. int.) *m/z* 232 (M^+, 21%), 109 (63), 91 (62), 81 (100), 53 (82); ^{1}H nmr ($CDCl_3$) δ 1.42 (3H, s, $C_{(14)}$-CH_3), 1.70 (3H, s, $C_{(15)}$-CH_3), 4.57 (1H, t, *J*=9.3 Hz, $C_{(6)}$-H), 4.74 (1H, d, *J*=9.8 Hz, $C_{(5)}$-H), 4.85 (1H, d, *J*=11.2 Hz $C_{(1)}$-H), 5.53 (1H, d, *J*=2.9 Hz, $C_{(13)}$-H), 6.26 (1H, d, *J*=2.9 Hz, $C_{(13)}$-H). Compound **2**, $C_{15}H_{18}O_2$, colorless needles, mp 59-60° (*n*-hexane/Me_2CO) [lit. mp 59-60° (2)]; $[\alpha]_D$ −12.6° (*c* 1.98, $CHCl_3$) [lit. −11.2° (*c* 1.1, $CHCl_3$) (2)]; ir max (KBr) 1770, 1650, 1420 cm^{-1}; eims (rel. int.) *m/z* 230 (M^+, 67%), 150 (95), 91 (100); ^{1}H nmr ($CDCl_3$) δ 3.96 (1H, t, *J*=9.3 Hz, $C_{(6)}$-H), 4.81 (1H, s, $C_{(14)}$-H), 4.90 (1H, s, $C_{(14)}$-H), 5.06 (1H, d, *J*=2.0 Hz, $C_{(15)}$-H), 5.26 (1H, d, *J*=2.0 Hz, $C_{(15)}$-H), 5.50 (1H,



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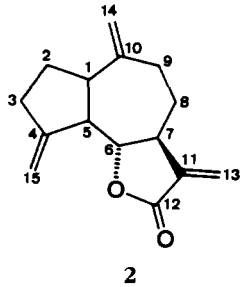

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d, J=3.4 Hz, $C_{(13)}$-H), 6.20 (1H, d, J=3.4 Hz, $C_{(13)}$-H).

RESULTS AND DISCUSSION

Preincubation (15 min) with a methanolic extract of the roots of *S. lappa* inhibited contractions induced by KCl (4×10^{-3} M) on rabbit-isolated aorta and had a reduced effect on contractions induced by norepinephrine (1×10^{-6} M). At the concentrations tested, the extract was without intrinsic activity on aortic tone. The bioassay-directed fractionation of the extract led to the isolation of two active compounds, **1** and **2**, in crystalline form. Both **1** and **2** were suggested to have an α-methylene-γ-lactone moiety on the basis of their ir and ^{1}H-nmr spectra, e.g., 1770, 1670, and 1440 cm^{-1}, 5.53 and 6.26 ppm in the ir and ^{1}H-nmr spectra of **1**, respectively. The molecular formulas of **1** ($C_{15}H_{20}O_2$) and **2** ($C_{15}H_{18}O_2$) were established by the molecular ion peaks in their mass spectra. The ^{1}H-nmr spectrum of **1** showed two methyl groups and two olefinic protons. On the other hand, three oxo methylene signals were observed in the ^{1}H-nmr spectrum of **2**. From the above data, **1** and **2** appear to be costunolide and dehydrocostus lactone, respectively, which were previously isolated as major constituents of the same species (3-6). Their identity was confirmed by comparison of their physicochemical properties with those in references (2-8).

As shown in Figure 1, **1** inhibited contractions of the aorta induced by, but exerted considerably less effect on those induced by norepinephrine, indicating the possible calcium antagonistic action of **1**. Compound **2** caused similar inhibitory effects on the aorta, but its specificity of KCl-induced contraction appeared to be less than that observed with **1**.

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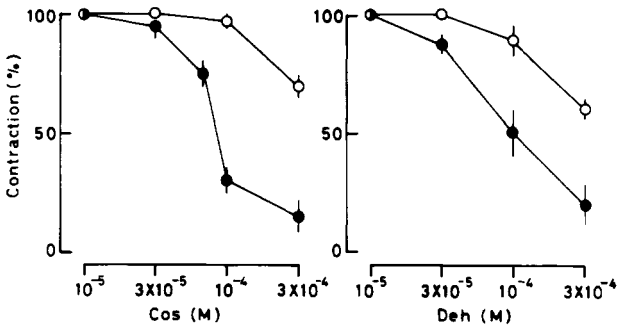

FIGURE 1. The inhibitory effect of constunolide (Cos) and dehydrocostus lactone (Deh) on KCl and norepinephrine-induced contractions of rabbit aorta. ○, 10^{-6} M norepinephrine; ●, 4×10^{-2} M KCl. The response to norepinephrine (10^{-6} M) or KCl (4×10^{-2} M) is expressed as 100%. Symbols and vertical bars indicate means ± s.e. of mean (n=6).

LITERATURE CITED

1. Y. Ohizumi and T. Yasumoto, *J. Physiol.*, **337**, 711 (1983).
2. S.B. Mathur, *Phytochemistry*, **11**, 449 (1972).
3. A.S. Rao, G.R. Kelkar, and S.C. Bhattacharyya, *Tetrahedron*, **19**, 275 (1960).
4. T. Ukita, *J. Pharm. Soc. Jap.*, **59**, 231 (1939).
5. L. Crabalona, *Bull. Soc. Chim. France*, **15**, 357, (1948).
6. Y.R. Naves, *Helv. Chim. Acta*, **31**, 1172 (1948).
7. V. Herout and F. Šorm, *Chem. Ind. (London)*, 1067 (1959).
8. M. Romanuk, V. Herout, and F. Šorm, *Chem. Listy*, **49**, 1879 (1955).

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