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BIOLOGICALLY ACTIVE CONSTITUENTS OF CENTIPEDA MINIMA: ISOLATION OF A NEW PLENOLIN ESTER AND THE ANTIALLERGY ACTIVITY OF SESQUITERPENE LACTONES

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Two pseudoguianolide sesquiterpenes were isolated as antiallergic constituents of Centipeda minima, which has been used as a folk medicine in southeast Asia. One of the sesquiterpenes, 6-O-senesioylplenolin (2), is a new pseudoguaianolide sesquiterpene lactone, which was isolated along with known pseudoguaianolide, arnicolide C (**1**). The two sesquiterpenes exhibited significant antiallergy activity in passive cutaneous anaphylaxis (PCA) tests. An amide isolated along with the sesquiterpenes was identified as aurantiamide acetate (3). Three flavonoids obtained from this plant also showed significant antiallergy activity.

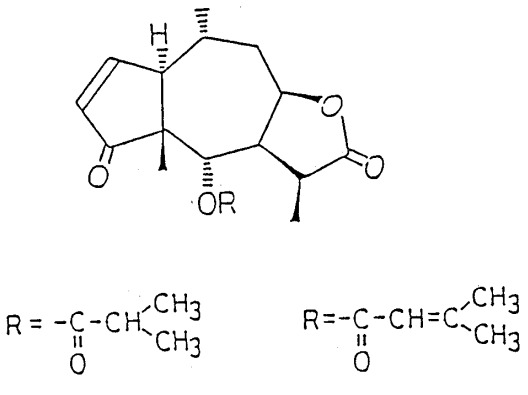
KEYWORDS——Centipeda minima; Compositae; sesquiterpene; arnicolide C; 6-O-senesioylplenolin; aurantiamide acetate; antiallergy; PCA; mast cell

As a part of our general interest in the isolation and characterization of biologically active compounds contained in medicinal plants used in Oriental medicine,[1] we have investigated Centipeda minima O.Kuntze (Compositae), which has been used as a remedy for cold, nasal allergy and asthma in southeast Asia.[2] Isolation of steroid and triterpenoids has been reported by Murakami et al. in their phytochemical study of this plant.[3] In the course of our screening study on oriental medicinal drugs, a hot aqueous extract of this plant exhibited a significant antiallergy activity in passive cutaneous anaphylaxis (PCA) tests, the most commonly used bioassay to evaluate antiallergy effects on Type I allergy.[4] The extract also showed potent inhibitory effects on histamine release from rat peritoneal mast cells induced by compound 48/80 or concanavalin A (con A).[5] The latter in vitro bioassay method is well correlated with the PCA test and has been used in studies of the biologically active constituents of medicinal plants.[5,6] A preliminary investigation revealed that the active constituents were soluble in organic solvents and plant material (5 kg) was extracted with chloroform followed by methanol. An n-hexane-soluble fraction of the chloroform extract (132 g) contained a large amount of volatile compounds and a separate gas chromatography-mass spectral analysis (GC-MS) showed that it contained heptan-2-ol, hepta-2,4-dien-1-ol, iso-butyric acid, benzylalcohol, cis-chrysanthenol, cis-

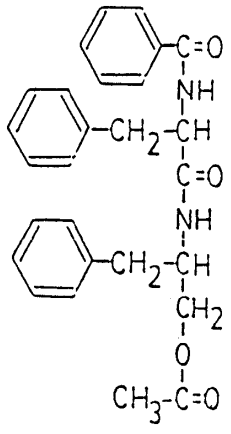
chrysanthenyl acetate, methyl linoleate, β-gurjunene, methyl palmitate, deca-2,4-dien-1-ol, ethyl palmitate, phytol, caryophyllane-2,6-β-oxide and dihydroactinidiolide. A methanol-soluble fraction of the chloroform extract (43 g) had a strong inhibitory effect (60% inhibition at 100 μg/mg) on histamine release from mast cells. The fraction was subjected to Sephadex LH-20 column chromatography and eluted successively with chloroform, acetone and methanol. A high inhibitory activity (82% inhibition at 75 μg/ml) was observed in a fraction eluted with chloroform. Repeated chromatographic separation of this fraction (30 g) on Sephadex LH-20, silica gel and Lobar(RP-8) columns along with the *in vitro* bioassay testing yielded three substances, compounds I-III.

Compound I (890 mg), $C_{19}H_{26}O_5$, showed IR and NMR spectra of typical sesquiterpene lactone and it was finally identified as arnicolide C (**1**) by direct comparison with an authentic sample isolated from a European medicinal plant *Arnica montana* L. (Compositae).[7,8] Compound II (450 mg), $C_{20}H_{26}O_5$, gave a ^{1}H-NMR spectrum very similar to that of arnicolide C (**1**) except for the signals arising from an ester group. The ^{1}H-NMR spectrum showed the signals of an ester group assignable to two methyl groups on olefinic carbon (δ 1.85 and 2.13, d, J=1.5) and an olefinic methine (δ 5.49, sept, J=1.5). This indicates that the ester residue is a senesioyl group. This is further supported by the Mass spectrum which gave a base peak corresponding to senesioyl ion at m/z 83. Thus compound II was identified as 6-O-senesioylplenolin (**2**).[9] Although nine plenolin (11,13-dihydrohelenalin) esters have been isolated from Compositae plants,[7,10] the senesioyl ester of plenolin is a new sesquiterpene isolated for the first time from a plant. Compound III (15 mg) was shown by elemental analysis and mass spectroscopy to contain the nitrogen atoms, $C_{27}H_{28}O_4N_2$. The ^{1}H-NMR spectrum revealed the presence of phenylalanine and phenylalanol moieties. All the spectral data of compound III were identical to those reported for aurantiamide acetate (**3**),[11] which had been isolated from the seeds of *Piper aurantiacum* Wall. (Piperaceae).[12]

The biological activity of arnicolide C (**1**), 6-O-senesioylplenolin (**2**) and aurantiamide acetate (**3**) is summarized in Table I. The occurrence of more than



arnicolide C (1) 6-O-senesioylplenolin (2)



aurantiamide acetate (3)

Table I. Inhibition of Histamine Release from Mast Cells and Antiallergy Effect in PCA Tests

	Inhibition of histamine helease[a] (IC_{50})	Inhibition of pigment leakage in PCA[b] (%)	
Arnicolide C(1)	3.0×10^{-5}M	61.6	44.4
6-O-Senesioylplenolin(2)	1.8×10^{-5}M	37.4	41.0
Aurantiamide acetate(3)	2.3×10^{-4}M	Not tested	

a) Histamine release was induced with con A in the presence of phosphatidylserine.
b) Oral administration at a dose of 50 mg/kg. Experiments were carried out with antiserum at two differen dilution ratios.

one thousand sesquiterpene lactones has been reported and a number of compounds attracted considerable attention due to their various biological activities such as antitumor activity, cytotoxicity, allergic contact dermatitis, and microbial and plant growth inhibition.[13] However this is the first time that antiallergy activity of sesquiterpene lactones was demonstrated by in vitro and in vivo bioassay tests. It is worth noting here that the two sesquiterpene lactones showed an antiallergy effect in PCA tests with oral administration at a dose of 50 mg/kg. Although inhibition of mast cell histamine release was not so prominent by aurantiamide acetate (**3**) as by the sesquiterpenes, it is of interest to test the biological activity of the related amide derivatives, since an amide developed from a medicinal plant constituent, 3,4-dimethoxycinnamoylanthranilic acid, is now used clinically for the treatment of asthma.[14]

In addition to compounds I-III, a fraction obtained from the Sephadex LH-20 column chromatography of the methanol soluble fraction of the chloroform extract afforded three flavonoids which inhibited mast cell histamine release. They were identified as quercetin 3,3'-dimethylether (45 mg),[15] quercetin 3-methylether (38 mg)[16] and apigenin (ca.5 mg). The IC_{50} values of the flavonoids in the inhibition of histamine release from mast cells were 1.0-0.5 X 10^{-5} M. They also showed significant effects in PCA tests by oral administration (50 mg/kg) and the inhibition ratios of pigment leakage were in a range of 39-67%. The antiallergy activity of the flavonoid was rigorously demonstrated for baicalein and quercetin with a guinea pig passive anaphylaxis model.[17] The effects of the flavonoids on mast cells and basophil leukocytes have been extensively studied because of their structural similarity to cromoglycate.[18] Quercetin 3,3'-dimethylether showed the most potent antiallergy effect in PCA tests with oral administration. The structure-activity relationships of related compounds are now under investigation.

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8) Arnicolide C (6-O-isobutyroylplenolin); mp 135-137'C(from methanol) [lit.137-138'C].[7] IR and NMR spectra were identical with the literature data.[7]
9) 6-O-Senesioylplenolin; mp 187-190°C(from methanol). High Resolution MS m/z: Calcd for $C_{20}H_{26}O_5$: 346.1777. Found: 346.1740. MS m/z: 346(M^+), 318, 263, 246, 321, 83(base peak). $[\alpha]_D^{24}$ -47.6° (c=0.0013, chloroform). UV λ_{max}^{EtOH} nm(log ε): 222(4.0). IR ν_{max}^{KBr} cm^{-1}: 1770, 1722, 1715, 1550. ^{1}H-NMR(in $CDCl_3$) :1.04 (15CH_3, s), 1.24 (14CH_3, d, J=6.8 Hz), 1.54 (13CH_3, d, J=7.6 Hz), 1.68 (9C-αH, ddd, J=2.0, 11.0, 15.4 Hz), 1.85, 2.13 (19 and 20-CH_3, dx2, J=1.5 Hz), 2.21 (10C-H, m), 2.46 (9C-βH, ddd, J=2.2, 6.1, 15.3 Hz), 2.91 (7C-H, dd, J=6.4, 10.3 Hz), 3.05-3.12 (1C-H, dq, J=10.3, 7.6 Hz), 4.75 (8C-H, ddd, J=6.4, 1.7, 6.1), 5.46 (6C-H, br s), 5.49 (17C-H, sept, J=1.5 Hz), 6.05 (3C-H, dd, J=3.2, 6.1 Hz), 7.66 (2C-H, dd, J=1.9, 6.0 Hz). ^{13}C-NMR (in $CDCl_3$) ppm: 11.1(q), 17.7(q), 19.8(q), 20.4(q), 25.9(d), 27.5(q), 40.5(d), 40.9(t),48.9(d), 54.0(d), 54.8(s), 70.9(d), 79.6(d), 115.3(d), 129.3(d), 158.0(s), 161.9(d), 164.8(s), 178.9(s), 209.3(s).
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11) Aurantiamide acetate; mp 188-190°C(from methanol). $[\alpha]_D^{30}$ -28.6° (c=0.0034, chloroform) [lit.-23.6°].[12] Anal. Calcd for $C_{27}H_{28}O_4N_2$: C,72.95; H,6.35; N,6.30. Found C,72.76; H,6.33; N,6.20. Ms, IR and NMR spectra were identical with the literature data.[12]
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