EUPONIN: A NEW EPOXY SESQUITERPENE LACTONE INHIBITING INSECT DEVELOPMENT FROM *EUPATORIUM JAPONICUM*[1)]

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Euponin, a new naturally occurring guaianolide inhibiting insect development, was isolated from the leaves of *Eupatorium japonicum* Thunb. (Compositae). Its structure was elucidated on the basis of chemical transformations and full spectral analysis.

As described previously,[2-4)] our novel "Drosophila test" proved to be a convenient bioassay method for searching insect development inhibitors in extracts of higher plants.

One of these extracts tested, the methanol extract of *Eupatorium japonicum* Thunb. was of special interest because of the observation that larvae of the fruit-fly could not grow in rearing medium containing the extract. This fact suggested that it contained growth inhibitors and/or antifeedants against the insect. We have examined the extract of *E. japonicum* and isolated a sesquiterpene lactone as an active principle.

In this communication we wish to report the isolation and

structure elucidation of the new epoxy sesquiterpene lactone, which we have named euponin.

Euponin $C_{20}H_{24}O_6$(I) (M^+, *m/z* 360), mp. 148-150°C, $[\alpha]_D^{24}$ -69° (c 1.0, EtOH), UV(EtOH) 210 nm(end absorption, ε=19000), was isolated from the methanol extract of *E. japonicum* leaves by successive solvent partitions and chromatography.

Euponin contains a hydroxyl group (3510 cm^{-1}) and an α-methylene-γ-lactone grouping (δ 6.2 and 5.5, 1H each, doublets, *J*=3 Hz; 1770 and 1650 cm^{-1}).

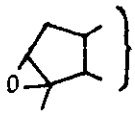
The ^{13}C-NMR spectrum[5] of euponin revealed that it has a tetracyclic structure.

The IR spectrum[5] of euponin also showed the presence of an ester carbonyl group (1700 cm^{-1}). The ^{1}H-NMR spectrum[5] displayed the typical signals of an angeloyl group (one olefin proton multiplet at δ 6.06, vinyl methyl multiplets at δ 1.76 and 1.90). The presence of the angeloyl function was further shown by the high-resolution mass spectrum, which had peaks corresponding to M-C_5H_7O and M-$C_5H_8O_2$, and a base peak at *m/z* 83 (C_5H_7O).

The ^{1}H-NMR spectrum of euponin showed 3H multiplets overlapped near δ 4. These signals were ascribed to two carbinyl protons and one lactonic proton. These carbinyl proton signals were shifted downfield by 0.38 ppm and appeared as a pair of doublets (δ 4.29 and 4.43, *J*=12 Hz) in the spectrum of the acetate (II) of euponin, suggesting that this carbinol is primary. Manganese dioxide oxidation of euponin gave an aldehyde (III), the ^{1}H-NMR spectrum (C_6D_6) of which exhibited a sharp singlet (δ 9.49) due

to an aldehyde proton, but no carbinyl proton signals in the region of δ 4. Its UV absorption (EtOH) λ_{max} 241 nm (ε=12600) suggested that the aldehyde group was conjugated with a tetrasubstituted carbon-carbon double bond. This suggestion was confirmed by its ^{1}H-NMR spectrum which displayed no olefinic protons other than those of the γ-lactone and angeloyl moiety. Hence, euponin is deduced to have a primary carbinol attached to a tetrasubstituted double bond.

Two ^{13}C-NMR signals due to two species of carbon carrying oxygen (δ 63.6, -O-CH and 66.6, -O-C-) suggested the presence of an oxide ring as the sixth oxygen function. A sharp 3H singlet (δ 1.70) and a broad 1H singlet (δ 3.41) seemed to be ascribed to the methyl and the proton attached to the oxide ring.



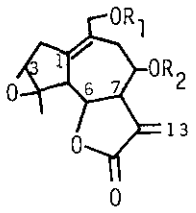
(A)

Inspection of literatures[6-9] revealed that a set of these signals were characteristic of the moiety (A) in some guaianolides, particularly these signals in berlandin (IV)[7] resembled those of euponin very closely in chemical shift and form. This regarded euponin as a guaianolide with C-3/C-4 epoxy grouping.

Location of angeloxy and the lactone ether oxygen on the seven membered ring remained to be solved. In the ^{1}H-NMR spectrum of euponin, irradiation at δ 3.1 (H-7) collapsed the multiplet at δ 5.7 (-CH-OAng) as well as the H-13a and H-13b doublets and the lactonic proton triplet. On the other hand, in the ^{1}H-NMR spectrum (C_6D_6) of III, irradiation of the proton (-CH-OAng) at

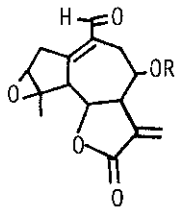
δ 5.4 collapsed the H-9 double doublet (δ 3.4, J=15 and 6Hz) to a doublet (J=15 Hz), and irradiation at δ 2.3 (H-9' and H-7) collapsed not only the H-13a and H-13b doublets to singlets but also the multiplet at δ 5.4 to a doublet (J=6 Hz). These facts indicate that the proton (-C<u>H</u>-OAng) is located at C-8.

The data mentioned above lead to the conclusion that the plane structure of euponin is represented by I.

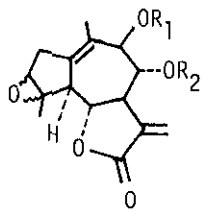


I, R_1= H; R_2=Angeloyl

II, R_1=Ac, R_2=Angeloyl



III, R=Angeloyl



IV, R_1=Angeloyl

R_2=Ac

In the ^{1}H-NMR spectrum of the acetate (II), the splitting pattern (triplet, J=10 Hz) of the lactonic proton signal could be clearly observed. The splitting pattern shows the *trans*-diaxial disposition of the protons at C-5(α), C-6(β) and C-7(α), a feature common to the most of the C-6 lactone in known guaianolides.[6-10]

The stereochemistry of euponin is under investigation.

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5 IR spectra were run in KBr pellets. ^{13}C-NMR spectra at 25.05 MHz and ^{1}H-NMR spectra at 60 MHz were taken in $CDCl_3$ containing tetramethylsilane as internal standard, unless otherwise specified.

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