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AN AMIDE FROM *SALMEA SCANDENS*

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Key Word Index—*Salmea scandens*; Compositae; Heliantheae; isobutylamides; costunolide.

Abstract—Costunolide and the isobutylamides of 2*E*,4*E*,8*Z*,10*Z*- and 2*E*,4*E*,8*Z*,10*E*-dodeca-2,4,8,10-tetraenoic acid were isolated from the roots of *Salmea scandens*.

The tropical American genus *Salmea* has not been investigated chemically. In the following, we describe the results of our study of its most common representative, *S. scandens* (L.) DC., which has various uses in indigenous medicine, apparently mainly as an anaesthetic ([1, 2]; Hernandez, J., personal communication).*

Examination of the above-ground parts of a small *S. scandens* collection from Puerto Rico gave no well-characterized substances. Extracts of the roots, which exert a numbing effect on the tongue, furnished, in addition to sitosterol, stigmasterol, linolenic and stearic acids, a small amount of costunolide (**1**) and two unstable isomeric compounds of formula $C_{16}H_{25}NO$ in approximately equal amounts. ^{1}H NMR spectroscopy (Table 1) identified one of these as *N*-isobutyl-2*E*,4*E*,8*Z*,10*E*-dodeca-2,4,8,10-tetraenamide (**2**), earlier found in *Spilanthes alba* [3]. In the ^{1}H NMR spectrum of the other compound, the signals of all the protons were also clearly distinguishable. Sequential decoupling showed that $J_{10,11}$ was 10 Hz instead of 15 Hz as in **2**; in addition, H-8 and H-9 showed downfield and H-10 and H-11 showed upfield shifts. Hence, the second substance was the previously unreported 10*Z*-isomer **3**. In view of the known physiological effects of herculin, pellitorine and similar amides [4–6], compounds **2** and **3** are undoubtedly responsible for the organoleptic properties of the roots.

The presence of identical polyunsaturated amides in *Spilanthes* and *Salmea* is of chemotaxonomic interest, since there is some question about the relationships between these two genera [7]. In the most recent treatment of Robinson [8], these two genera, as well as *Heliopsis*, *Echinacea* and *Acmella*, which also give rise to polyunsaturated amides, are placed in the same subtribe, Ecliptinae.

*Its use against toothache is illustrated by such common names as 'duerme boca' (Colombia [1] and 'Bejuco de muela' (Puerto Rico) (Hernandez, J., personal communication).

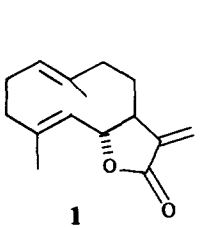
EXPERIMENTAL

Air-dried aerial parts of *S. scandens* (500 g), collected in 1982 by César Xavier Hernandez in the vicinity of Rio Piedras, Puerto

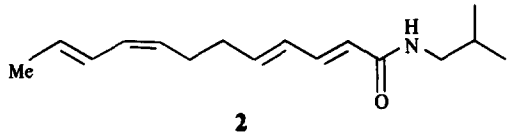
Table 1. ^{1}H NMR spectral data of compounds **2** and **3** ($CHCl_3$, 270 MHz)

H	2	3
2	5.80 *d* (15)	5.77 *d* (15)
3	7.20 *dd* (15, 10)	7.19 *dd* (15, 10)
4	6.20 *dd* (15, 10)	6.19 *dd* (15, 10)
5	6.10 *dt* (15, 7)	6.09 *dt* (15, 7)
6a,b	2.28 *m*	2.26 *m*
7a,b	2.28 *m*	2.26 *m*
8	5.26 *dt* (10, 7)	5.42 *dt* (10, 7)
9	6.00 *brt* (10)	6.32 *brt* (10)
10	6.32 *br dd* (15, 10)	6.26 *tq* (10, 7)
11	5.72 *dq* (15, 7)	5.55 *dq* (10, 7)
12	1.80 *br d* (7)	1.75 *dd* (7, 2)
1'a,b	3.16 *t* (7)	3.16 *t* (7)
2'	1.80 *m*	1.80 *m*
3', 4'	0.94 *d* (7)	0.93 *d* (7)

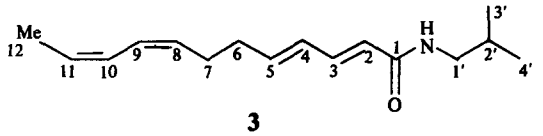
Coupling constants (*J* in Hz) are given in parentheses.



1



2



3

Rico, were extracted continuously with $CHCl_3$ for 3 days and worked up in the usual fashion [9]. TLC of the crude gum (5 g) indicated the presence of a large number of relatively non-polar substances, each in rather small amount, which could not be characterized satisfactorily. Extraction of the roots (250 g) gave 3.5 g of crude gum which was absorbed on 5 g silicic acid (Mallinckrodt, 100 mesh) and chromatographed over 200 g of the same absorbent packed in hexane, 250 ml fractions being collected as follows: 1–2 (hexane), 3–6 (hexane–EtOAc, 19:1) 7–10 (hexane–EtOAc, 9:1), 11–14 (hexane–EtOAc, 4:1), 15–18 (hexane–EtOAc, 3:2), 19–22 (hexane–EtOAc, 2:3), 23–26 (hexane–EtOAc, 1:4), 27–28 (EtOAc), 29–30 (EtOAc–MeOH, 19:1) and 31–32 (EtOAc–MeOH, 9:1). Fraction 8 upon purification by TLC (C_6H_6–EtOAc, 9:1) gave 15 mg costunolide. Fraction 9 on standing in hexane–EtOAc deposited 22 mg of a mixture of sitosterol and stigmasterol. Fraction 12 showed two spots; separation by prep. TLC (C_6H_6–EtOAc, 9:1) yielded 70 mg of a mixture of linolenic and stearic acids as the less polar material. The lower band on purification by TLC (7% $AgNO_3$–silica gel, C_6H_6–EtOAc, 9:1, several developments) furnished the unstable amides **2** (22 mg) and **3** (24 mg). ^1H NMR spectral data are reported in Table 1. Amide **3** had IR bands ($CHCl_3$ at 3400 br, 1670 and 1670 cm^{-1}); the low-resolution MS exhibited peaks at *m/z* (rel. int.): 247 $[M]^+$ (1.9), 167 (25.5) and 81 (100).

Acknowledgements—We wish to thank Mr. César Xavier Hernandez for the collection and Dr. José Hernandez for bringing the problem to our attention.

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IDENTIFICATION AND DISTRIBUTION OF ONONITOL IN NODULES OF *PISUM SATIVUM* AND *GLYCINE MAX*

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Key Word Index—*Pisum sativum*; *Glycine max*; Leguminosae; pea; soybean; cyclitol; 4-*O*-methyl-*myo*-inositol.

Abstract—Ononitol (4-*O*-methyl-*myo*-inositol) was identified as a major carbohydrate in *Pisum sativum* nodules, comprising 25–34% of the total mono- plus disaccharides in nodules formed by two *Rhizobium leguminosarum* strains. Ononitol was purified from *Glycine max* nodules and was found to be a minor carbohydrate in these nodules. The distribution of ononitol in bacteroids and cytosol from soybean nodules suggests that it is not synthesized by bacteroids.

In the analysis of TMSi derivatives of carbohydrates from pea (*Pisum sativum*) nodules by GC, a major peak was found with a retention time very similar to that of sequoyitol (5-*O*-methyl-*myo*-inositol) using a column of 3% OV-17 and a temperature programme of 150° (8 min hold) increasing at 5°/min to 240°. However, the retention time of the unknown, relative to penta-TMSi-β-phenyl-glucose (internal standard), was consistently 0.02 units different from the RR_t of TMSi sequoyitol.

The unknown was a neutral compound as indicated by its failure to bind to columns of Dowex 50-H^+ or Dowex 1-formate. The unknown was hydrolysed only under harsh conditions (3 M HCl, 105° for several hr). Hydrolysis for increasing periods of time (1–13 hr) gave increasing amounts of a compound, the TMSi derivative of which co-chromatographed precisely with TMSi *myo*-inositol on OV-17. The decrease in unknown was proportional to the increase in *myo*-inositol.

The neutrality and remarkable stability of the unknown and the hydrolytic conversion of the unknown to *myo*-inositol indicated that the compound was an *O*-methyl-cyclitol in the *myo*-inositol family. Only three *O*-methyl