Phytochemistry, Vol. 25, No. 10, pp. 2289–2290, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

A MOSQUITO LARVICIDE IN *SPILANTHES MAURITIANA*

ISAAC J. O. JONDIKO

The International Centre of Insect Physiology and Ecology, P.O. Box 30772, Nairobi, Kenya

(*Revised received* 10 *February* 1986)

Key Word Index—*Spilanthes mauritiana*; Compositae; *Aedes aegypti*; mosquito larvicide; *N*-isobutylamide.

Abstract—The methanol extract of fresh vegetative aerial parts of *Spilanthes mauritiana* afforded, after repeated chromatographic separations and mosquito larvicidal bioassays, a potent mosquito larvicide *N*-isobutyl-2*E*,4*E*,8*E*,10*Z*-dodeca-2,4,8,10-tetraenamide. The structure of the compound followed from spectroscopic considerations. It gave 100% mortality against third instar larvae of *Aedes aegypti* at 10^{-5} mg/ml.

INTRODUCTION

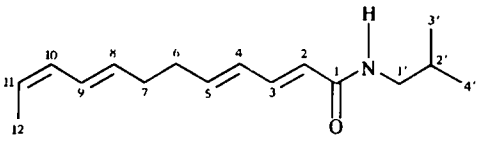
In the course of our search for insecticidal compounds from tropical plants, *Spilanthes mauritiana*, a Compositae, was collected from Kisii Highlands in Kenya for screening against mosquito larvae. This plant is traditionally used for treatment of toothache and diarrhoea and also for the control of Anopheles mosquito [1, 2]. Several mosquito larvicidal compounds have been isolated from *Spilanthes* species such as *S. oleracea* [3], *S. alba* [4], *S. americana* [5], *S. acmella* [6] and *S. ocymyfolia* [7] but none from *S. mauritiana*.

RESULTS AND DISCUSSION

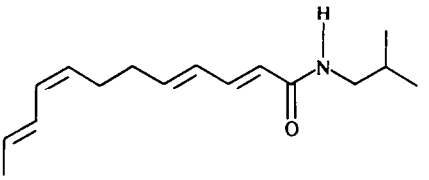
Repeated chromatographic separations of the methanol extract of fresh aerial parts of *Spilanthes mauritiana* coupled with larvicidal bioassay [8] of the fractions led to the isolation of a homogeneous pale yellow oil whose structure was elucidated from spectroscopic data (^{1}H NMR, ^{13}C NMR, MS, IR and UV).

The low resolution mass spectrum gave a molecular ion at *m/z* 247 assigned to $C_{16}H_{25}NO$ and the fragmentation pattern observed favoured structure **1**. The most easily cleaved bond was the allylic bond, C-6–C-7 and its cleavage led to the parent base peak observed at *m/z* 81 and another major ion observed at *m/z* 167. The NH group was deduced from the IR signal at 3295 cm^{-1} and a broad resonance at δ5.6 in the ^{1}H NMR spectrum which disappeared on D_2O exchange. The UV absorption at 260 nm and the IR peaks at 1550 and 1650 cm^{-1} were attributed to the double bond conjugated amide group. The ^{1}H NMR shifts were assigned with reference to those published for compounds **2** [9] and **3** [10] and with consecutive irradiation experiments. The 10-*cis* geometry was deduced from the observation that shifts for H-10 and H-11 were at higher fields than those recorded for the corresponding protons for **2** and also that $J_{10,11}$ was found to be 10 Hz instead of 15 Hz, expected for a *trans*-double bond. The 8-*trans* geometry was also apparent from the lower field shifts for H-8 and H-9 than those recorded for **2** and also from the $J_{8,9}$ found to be 15 Hz.

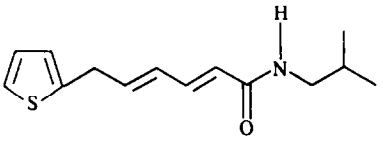
The assignment of ^{13}C NMR resonances was done with reference to signals assigned for **3** [10] and 2*Z*-hept-2-ene [11]. The 2*E* and 4*E* geometries were confirmed from the



1



2



3

^{13}C NMR shifts which were close to those recorded for **3**. The 10*Z* double bond was confirmed by the observation that the carbon of the olefinic methyl resonated at δ13.13 which was comparable to that recorded for 2*Z*-hept-2-ene. Moreover, inspection of the ^{13}C NMR data [12] published for *N*-isobutyl-2*E*,4*E*,8*Z*,10*E*-dodeca-2,4,8,10-tetraenamide, *N*-isobutyl-2*E*,4*E*,8*Z*,10*Z*-dodeca-2,4,8,10-tetraenamide and *N*-isobutyl-2*E*,4*E*,8*E*,10*E*-dodeca-2,4,8,10-tetraenamide revealed that the ^{13}C NMR data for **1** did not correspond to any of these compounds. Hence this is the first time this isomer has been isolated.

The bioassay results (Table 1) indicated that the larvicidal activity increased with purification. The aqueous extract was devoid of activity while the chloroform extract showed 100-fold efficacy over the methanol extract and

Table 1. Mosquito larvicidal activity of the extracts

Test solutions	Dose (mg/ml)	Percentage mortality
Control	0.0	0
MeOH extract	0.05	100
$CHCl_3$ extract	1.0×10^{-4}	100
Compound 1	1.0×10^{-5}	100

compound 1 had ten-fold the activity of the chloroform extract. This high larvicidal activity is comparable to activities published for other *N*-isobutylamides [13].

EXPERIMENTAL

Aerial parts of *Spilanthes mauritiana* were collected from Kisii Highlands in Kenya in January 1983 and a specimen was deposited with the herbarium of the University of Nairobi, Kenya. The fresh leaves and stems (4 kg) were blended and soaked in MeOH (4 l.) for 1 week. A portion of the MeOH extract was set aside for larvicidal bioassay while the rest was evaporated *in vacuo* to 500 ml. This aq. residue was extracted with $CHCl_3$ (3 × 200 ml) to obtain an aq. extract (30 g) after freeze drying and a $CHCl_3$ extract (10 g) after vacuum evaporation. The two extracts were bioassayed for larvicidal activity. The $CHCl_3$ extract (9 g) was then subjected to silica gel chromatography using 30% EtOAc in hexane and collecting 300 ml fractions to obtain a potent larvicidal fraction three (0.1 g). This fraction was further purified using semi-prep. HPLC involving a reverse phase column (MCH-10, 50 × 0.8 cm) and MeCN as the mobile phase to obtain fraction three as a pale yellow oil (25 mg); IR ν_{max}^{Nujol} cm^{-1}: 3295, 3075, 1550, 1660, 995; UV λ_{max}^{EtOH} nm (ε 30 000); MS 70 eV, *m/z* (rel. int): 247 $[M]^+$ (0.02), 246 $[M-H]^+$ (0.03), 205 $[M-C_3H_6]^+$ (0.07), 167 $[M-C_6H_8]^+$ (28), 100 $[M-C_{11}H_{15}]^+$ (10), 81 $[M-C_{10}H_{16}NO]^+$ (100), 67 $[M-C_{11}H_{18}NO]^+$ (15), 41 $[M-C_{13}H_{20}NO]^+$ (32); ^{1}H NMR ($CDCl_3$): δ5.76 (*d*, H-2, J = 15 Hz), 7.15 (*dd*, H-3, J = 15, 10 Hz), 6.14 (*dd*, H-4, J = 15, 10 Hz), 6.07 (*dt*, H-5, J = 15 Hz), 2.25 (*m*, H-6, H-7, 4H) 6.33 (*dt*, H-8, J = 15, 7 Hz), 6.26 (*dd*, H-9, J = 15, 10 Hz), 5.62 (*dd*, H-10, J = 10, 7 Hz), 5.45 (*dq*, H-10, J = 10, 7 Hz), 1.75 (*d*, H-12, 3H, J = 7 Hz), 5.60 (*br s*, NH), 3.14 (*t*, H-1', 2H, J = 7 Hz), 1.80 (*m*, H-2'), 0.83 (*d*, H-3', H-4', 6H, J = 7 Hz); ^{13}C NMR ($CDCl_3$): δ166.55 (*s*, C-1), 122.45 (*d*, C-2), 141.17 (*d*, C-3), 129.55 (*d*, C-4), 141.96 (*d*, C-5), 33.00 (*t*, C-6), 26.97 (*t*, C-7), 124.50 (*d*, C-8), 128.94 (*d*, C-9), 130.21 (*d*, C-10), 124.37 (*d*, C-11), 13.12 (*q*, C-12), 47.17 (*t*, C-1'), 28.83 (*d*, C-2'), 19.90 (*q*, C-3' and C-4').

Mosquito larvicidal bioassay. Ten third instar larvae of *Aedes aegypti* were introduced into a 50 ml beaker containing 0.1 ml of the test soln in Me_2CO and 19.9 ml of H_2O. For a control experiment 0.1 ml of Me_2CO was used instead of the test soln. The larvae were then scored for percentage mortality after 24 hr. The experiment for each test soln and control soln was repeated × 4 (Table 1).

Acknowledgements—The author thanks Professor Thomas R. Odhiambo, the Director of International Centre of Insect Physiology and Ecology (ICIPE) for permission to publish this paper. Sincere thanks are also extended to Dr. Wilber Lwande and Professor Michael Bentley of the University of Maine at Orono, Orono, ME 04469, U.S.A., for ^{1}H NMR and ^{13}C NMR data.

REFERENCES

1. Kokwaro, J. O. (1976) *Medicinal Plants of East Africa*, p. 71. East Africa Literature Bureau, Nairobi.
2. Jacobson, M. (1975) *USDA Agr. Handbook*, p. 461. Washington, DC.
3. Jacobson, M. (1957) *Chem. Ind.* 50.
4. Krishnaswamy, N. R., Prasana, S., Sheshadri, T. R. and Vedantham, T. N. C. (1975) *Phytochemistry* **14**, 1666.
5. Jacobson, M. (1956) *J. Am. Chem. Soc.* **78**, 5084.
6. Bohlmann, F., Ziesche, J., Robinson, H. and King, R. M. (1980) *Phytochemistry* **19**, 2535.
7. Borges-del-Castillo, J., Vazquez-Bueno, P., Secundino-Lucas, M., Martinez-Martir, A. I. and Joseph-Nathan, P. (1984) *Phytochemistry* **23**, 2671.
8. World Health Organization Technical Report (1970) Ser. No. 443, p. 66.
9. Herz, W. and Kulanthaivel, P. (1985) *Phytochemistry* **24**, 173.
10. Greger, H. and Hofer, O. (1984) *Phytochemistry* **23**, 1173.
11. Johnson, L. F. and Jankonwski, W. C. (1972) *Carbon-13 NMR Spectra*, p. 264. John Wiley, New York.
12. Yasuda, I., Takeya, K. and Itokawa, H. (1981) *Chem. Pharm. Bull.* **29**, 564.
13. Kubo, I., Matsumoto, T., Clocke, J. A. and Kamikawa, T. (1984) *Experientia* **40**, 340.