4 The gorgonian collected in the bay of Naples was obtained from the supply Department of the Zoological Station (Naples) to whom the authors express their thanks.
5 Bergmann, W., and Burke, D.C., J. org. Chem. *20* (1955) 1501.
6 Baker, D.C., Haskell, T.H., Putt, S.R., and Sloan, B., J. med. Chem. *22* (1979) 273.
7 Waller, G.R., Biochemical applications of mass spectrometry, p. 759. Wiley-Interscience, 1972.
8 De Clercq, E., Krasewska, E., Descamp, J., and Torrence, D.F., Molec. Pharmac. *13* (1977) 980.
9 Pizer, L.I., and Cohen, S.S., J. biol. Chem. *235* (1960) 2387.
10 Cohen, S.S., in: Progress in the nucleic acid research and molecular biology, vol. 5, p. 1. Eds N.Y. Davidson and W.E. Cohen. Academic Press, New York 1966.
11 Lee, W.W., Benitez, A., Goodman, L., and Baker, B.R., J. Am. chem. Soc. *82* (1960) 2648; Reist, E.J., Benitez, A., Goodman, L., Baker, B.R., and Lee, W.W., J. org. Chem. *27* (1962) 3274.
12 Shannon, W.M., and Shabel, F.M., Jr, Pharm. Theor. *11* (1980) 263.
13 Whitley, R.T., Soong, S., Dolin, R., Galasso, G.J., Ch'ien, R.T., and Alford, C.A., N. Engl. J. Med. *297* (1977) 289.
14 Baker, D.C., Haskell, T.H., and Putt, S.R., J. med. Chem. *21* (1979) 1218.

0014-4754/84/040339-02$1.50 + 0.20/0

Molluscicidal and insecticidal activities of isobutylamides isolated from *Fagara macrophylla*

I. Kubo, T. Matsumoto, J.A. Klocke and T. Kamikawa[1]

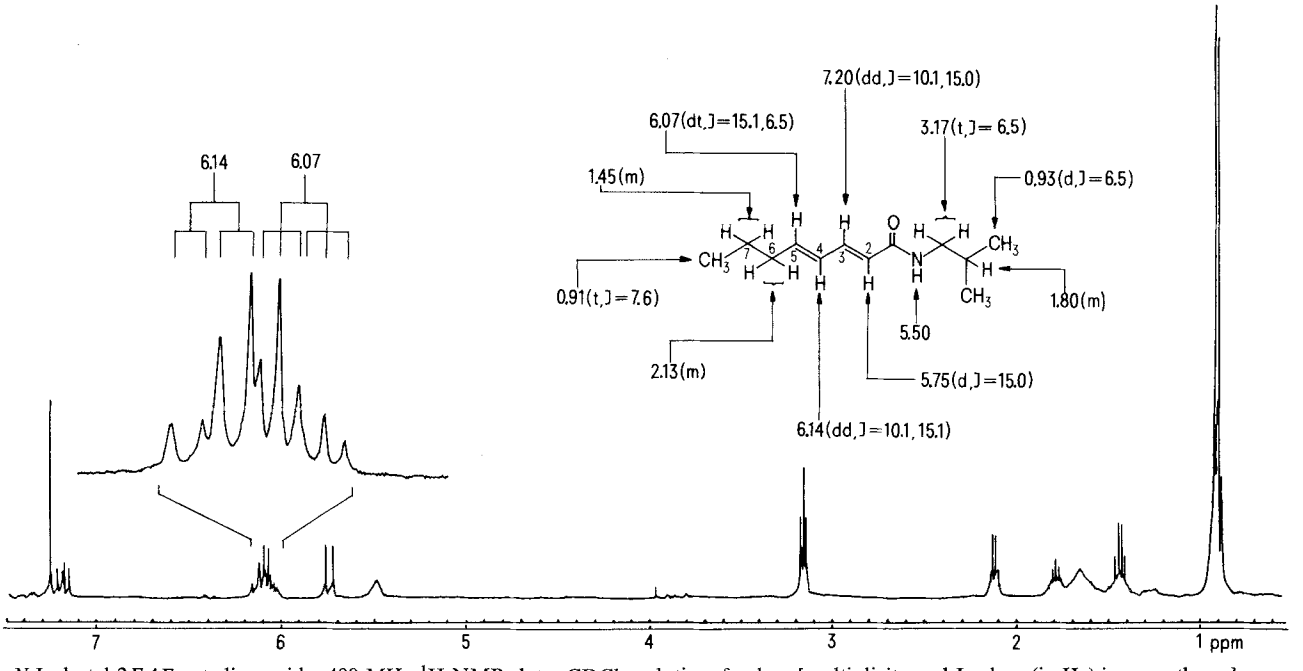
Division of Entomology and Parasitology, College of Natural Resources, University of California, Berkeley (CA 94720, USA), and Department of Chemistry, Faculty of Science and Technology, Kinki University, Kowakae, Higashi-Osaka-shi, Osaka 577 (Japan), 18 May 1983

Summary. Five isobutylamides were isolated as insect growth inhibitors and toxicants from *Fagara macrophylla* and identified from their spectroscopic data.

The East African medicinal tree *Fagara macrophylla* (Rutaceae) is known to be relatively free from insect attack. In order to test for chemical factors involved in this observed resistance, extracts of the *F. macrophylla* bark were incorporated into artificial diets optimized for several economically-important agricultural pest insects, pink bollworm *(Pectinophora gossypiella)*, tobacco budworm *(Heliothis virescens)*, corn earworm *(H. zea)* and fall armyworm *(Spodoptera frugiperda)*[2,3]. This led to the isolation of 5 insect growth inhibitors and/or toxicants which have been identified as fagaramide [*N*-isobutyl-3-(3,4-methylenedioxyphenyl)-2*E*-propenamide] (**1**), piperlonguimine [*N*-isobutyl-5-(3,4-methylenedioxyphenyl)-2*E*,4*E*-pentadienamide] (**2**)[4,5], 4,5-dihydropiperlongumine [*N*-isobutyl-5-(3,4-methylenedioxyphenyl)-2*E*-pentenamide] (**3**)[6], pellitorine (*N*-isobutyl-2*E*,4*E*-decadienamide) (**4**)[7], and *N*-isobutyl-2*E*,4*E*-octadienamide (**5**)[8] based on spectroscopic data.

The most abundant of the isolated amides was fagaramide **1**[9]. The structure of fagaramide has long been known[10], but the geometry of its side chain double bond has not been clearly established. This has now been confirmed as *trans* based on the large coupling constant (16 Hz) in the 400 MHz ^{1}H-NMR spectrum.

The ^{1}H-NMR spectra were more important in assigning the stereochemistry of conjugated double bond in **2**, **4** and **5**. For example, assignment of the geometry of *N*-isobutyl-2*E*,4*E*-octadienamide **5** was achieved as follow. The ^{1}H-NMR spectrum of **5** is reproduced in the figure along with an expanded representation of a part of the olefinic proton region. Irradiation of the 3-H doublet of doublets at 7.20



N-Isobutyl-2*E*,4*E*-octadienamide, 400 MHz ^{1}H-NMR data, $CDCl_3$ solution, δ values [multiplicity and J values (in Hz) in parentheses].

Table 1. Insect growth inhibitory activity (ED_{50}[a] in ppm) of natural isobutylamides

Compounds	Insect species (1st-instar) *Pectinophora gossypiella*	*Heliothis virescens*	*H. zea*	*Spodoptera frugiperda*
Fagaramide	440	350	510	530
Piperlongumine	430	370	–	500
4,5-Dihydropiperlongumine	800	–	–	1700
Pellitorine	15[b]	270	210	230
N-Isobutyl-2*E*,4*E*-octadienamide	70[c]	600	600	280

[a] ED_{50}-values are the effective doses for 50% growth inhibition. [b] LD_{90}-value, the lethal dose for 90% death, for pellitorine against *P. gossypiella* is 25 ppm. [c] LD_{90}-value, the lethal dose for 90% death, for *N*-isobutyl-2*E*,4*E*-octadienamide against *P. gossypiella* is 100 ppm. No lethal effect was observed by any of the compounds to 200 ppm against the other tested insect species.

Table 2. Lethal activity (LD_{100}[a] in ppm) of natural isobutylamides[b] against third-instar larvae of *Culex pipiens*

Compounds	LD_{100} (ppm)
Fagaramide	15
Piperlongumine	10
Pellitorine	5
N-Isobutyl-2*E*,4*E*-octadienamide	15

[a] LD_{100}-values are the lethal doses for 100% death in a 48-h bioassay. [b] 4,5-Dihydropiperlongumine was not tested due to insufficient quantities available.

Table 3. Lethal activity (LD_{50}[a] in ppm) of natural isobutylamides against the snail *Biomphalaria glabratus*

Compounds	LD_{50} (ppm)
Fagaramide	200
Piperlongumine	> 200
Pellitorine	> 200
N-Isobutyl-2*E*,4*E*-octadienamide	200

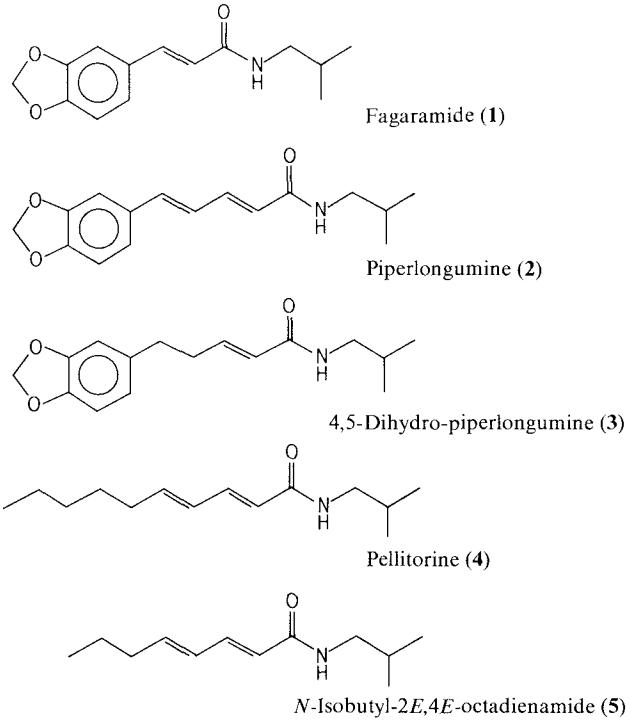
[a] LD_{50}-values are the lethal dose for 50% death.

ppm (J = 10, 15 Hz) changed the 2-H doublet at 5.75 ppm into a singlet, and also simplified the complex multiplet at 6.0–6.2 ppm. Thus, the structure can now be assigned as in **5**.

The artificial diet feeding assay mentioned above was employed to monitor the chromatographic separation of the 5 bioactive principles. Once separated, purified, and spectrally identified, the active principles were tested in the same artificial diet feeding assay in order to obtain ED_{50}-values, the effective doses for 50% growth inhibition. The growth-inhibitory activity of the 5 amides on 4 species of lepidopteran larvae of agricultural importance is shown in table 1. Pellitorine, the second most abundant amide isolated from *F. macrophylla*, was the most active of the isolated amides, especially against *P. gossypiella* (ED_{50} = 15 ppm). Pellitorine also caused death (LD_{90} = 25 ppm) to *P. gossypiella* larvae, but not to those of *H. zea*, *H. virescens* and *S. frugiperda*. The isolated compound that was closely related to pellitorine, *N*-isobutyl-2*E*,4*E*-octadienamide, also caused mortality to *P. gossypiella* only (LD_{90} = 100 ppm).

Additional bioassays were conducted with the house mosquito, *Culex pipiens*, and the freshwater snail, *Biomphalaria glabratus*, both aquatic species of medical importance. The lethal activity of 4 of the amides on *C. pipiens* is shown in table 2. The amides were dissolvee in 0.1% acetone in distilled water to give concentrations of 1–20 ppm. Third-instar *C. pipiens* were transferred (5 larvae/10 ml test solution) into 1-oz. plastic cups using a 1 × 1-inch circle of ordinary window screen. Each treatment was replicated 4 times and the minimum concentration of each compound which caused 100% mortality (LD_{100}) within 48 h at 25 °C and 16 L/8 D photoperiod was determined. In a result similar to that found with the artificial diet bioassay with lepidopterous larvae, pellitorine proved to be the most toxic of the assayed amides (LD_{100} = 5 ppm).

The lethal activity of the same amides on *B. glabratus* is shown in table 3. Molluscicidal activity was monitored as described previously[11]. In the case of molluscicidal activity fagaramide and *N*-isobutyl-2*E*,4*E*,-octadienamide show the best activity.



1 Insects were kindly supplied by the agencies of the USDA in Tifton, GA; Phoenix, AZ and Brownsville, TX. The authors thank Professor D. Heyneman for *B. glabratus* supply. This work was partly supported by a PHS Biomedical Grant to I. K.

2 Chan, B.G., Waiss, A.C. Jr, Stanley, W.L., and Goodban, A.E., J. econ. Ent. *71* (1978) 366.
3 Kubo, I., and Klocke, J.A., in: Plant Resistance to Insects: ACS Symposium Series 208, p. 329. Ed. P.A. Hedin. American Chemical Society, Washington, D.C., 1983, and references therein.
4 Chatterjee, A., and Dutta, C.P., Tetrahedron Lett. *1966*, 1797.
5û Chatterjee, A., and Dutta, C.P., Tetrahedron *23* (1967) 1769.
6 Loder, J.W., Moorhouse, A., and Russell, G.B., Aust. J. Chem. *22* (1969) 1531.
7 Crombie, L., J. chem. Soc. *1955*, 999.
8 Jacobson, M., J. Am. chem. Soc. *78* (1965) 5084.
9 Fish, F., and Waterman, P.G., Phytochemistry *11* (1972) 3007.
10 Thoms, H., and Thumen, F., Chem. Ber. *44* (1911) 3717.
11 Nakanishi, K., and Kubo, I., Israel J. Chem. *16* (1978) 28.

0014-4754/84/040340-02$1.50 + 0.20/0