Pure & Appl. Chem., Vol. 66, Nos 10/11, pp. 2347–2352, 1994.
Printed in Great Britain.
© 1994 IUPAC

Biologically active constituents of tropical and subtropical plants

W. Kraus, M. Köll-Weber, R. Maile, T. Wunder, and B. Vogler

Department of Chemistry, University of Hohenheim, 70593 Stuttgart, Germany

Abstract: Methanol extracts of a number of tropical and subtropical plants, such as *Ailanthus altissima, Acanthospermum hispidum, Heritiera littoralis*, and seed cake of *Azadirachta indica* ("neem cake"), have been tested for antifeedant, insecticidal, insect growth regulating (IGR), nematicidal, bactericidal, and fungicidal activity. Antifeedant activities towards *Epilachna varivestis* were observed with the *Ailanthus, Acanthospermum, Heritiera*, and neem cake extracts, resp., IGR activities were found in addition with *Ailanthus* and *neem cake* extracts. Bactericidal and fungicidal effects were observed with extracts from *A. hispidum*. We report on isolation, structure, and biological activity of some of the active principles.

In connection with our search for botanical pesticides we have investigated the constituents of the following plants:

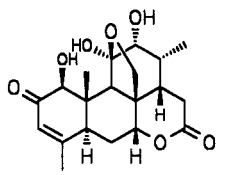
Acanthospermum hispidum (Melampodinae):	Aerial parts, collected in Zimbabwe
Ailanthus altissima (Simaroubaceae):	Seeds, collected in South Africa
Azadirachta indica (Meliaceae):	Seed cake ("neem cake"), collected in India
Heritiera littoralis (Sterculiaceae):	Bark, collected in Kenia

The air dried plant material was extracted with methanol at room temperature and worked up chromatographically. The antifeedant tests (dual choice tests), and the IGR tests (no choice test arrangements) were carried out with *Epilachna varivestis* larvae, according to the procedure described earlier [1], the tests for nematicidal acitivty with *Meloidogyne incognita*, the bactericidal tests with *Bacillus subtilis* and *Pseudomonas stutzeri,* and the fungicidal tests with *Cladosporium cucumerinum.*

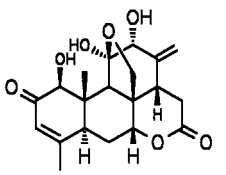
In preliminary screenings good feeding inhibition was observed with *Ailanthus* [2] - [4], *Acanthospermum, Heretiera,* and neem cake extracts, IGR activity was observed with *Ailanthus* [4], and neem cake extracts which are reported also to possess nematicidal properties [5], [6]. On topical application the crude *Heritiera* extract caused 20% mortality at a dosis of 15 μl of a 15% solution in acetone.

From the ***Ailanthus*** seeds two different extracts were prepared and tested: The petrol ether extracts (0.1%) on application by feeding to *E. varivestis* larvae caused 70% mortality of the adult beetles already after treatment for 4 days. The number of dead pupae was rather low (10%). Obviously with this extract the IGR activity is more important than the antifeedant activity. Somewhat different results were obtained with a 0.0125% methanol extract. Mortality of the adult beetles after 4 days treatment was only 40%, after 7 days treatment, however, no larvae or beetles survived.

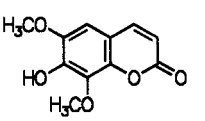
The main components of the methanol extract showing biological activity were identified to be chaparrinone **(1)**, and ailanthone **(2)**. Chaparrinone **(1)** has been shown earlier to posses insect antifeedant activity [2], [3]. In our experiments ailanthone **(2)** turned out to be a potent antifeedant, too, but seems to be also an insect growth inhibitor at lower concentration [4]. This observation will be investigated further.



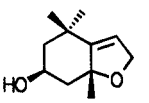
1



2



3



4

Chromatographic work up of the methanolic *H. littoralis* extract yielded Isofraxidin **(3)**, identified for the first time as a constituent of *H. littoralis*. The structure was determined on the basis of the 250 MHz nmr, mass, uv, and ir spectra, and by comparison of the spectral data with published values [7], [8]. **(3)** turned out to be a very potent antifeedant towards *E. varivestis* (100% feeding inhibition at 100 ppm).

The methanolic *Acanthospermum hispidum* extract yielded, on flash chromatography followed by HPLC, loliolide **(4)**, and 8 new biologically active sesquiterpene lactones **(5c, 6b, 6c, 7c, 9b, 9c, 10b, 12)** along with acanthospermal B **(8)** [9] and other known constituents **(5a/b, 6a, 7a/b, 9a, 10a, 11)** [10], [11].

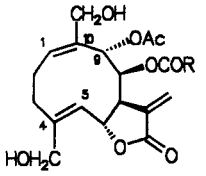
The structures of the new compounds were determined by spectroscopic means (^{1}H nmr, ^{13}C nmr, H,H COSY, nOe difference spectra, EIMS), and by comparing the spectral data with those of related known compounds. Position and *E*-configuration of the C-9/C-10 double bond in epoxide **(12)** was established on the basis of the following observations:

a) Absence of the typical spin pattern of 1-H (broad doublet of dubletts), indicating that 1-H is located adjacent to a methylene group.
b) Nuclear Overhauser effects 9-H → 8-H, 14-H; in systems having an *E*-configurated C-1/C-10 double bond as in compounds **(7)** nOe's 1-H → 14-H are observed instead.
c) The downfield shift of the 9-H signal, indicating that 9-H is an olefinic proton, which was identified by H,H COSY experiments carried out for the spin system 8-H/9-H.

The C-4/C-5 epoxy moiety was identified on the basis of the upfield shift of the 6-H and particularly the 5-H signals which were identified by spin decoupling experiments. The configuration at C-4, C-5, and C-6 of the epoxide **(12)** was assigned as 5-H_α, 6-H_β on the basis of the the known α-configuration of 7-H in acanthospermolides [12], the strong n.O.e. 5-H → 7-H, 15-H, and the coupling constants $J_{5,6}$ = 11.2 Hz and $J_{6,7}$ = 9.8 Hz which indicate *trans* configuration for 5-H/6-H and 6-H/7-H, respectively. Atomic distances, bond angels, and coupling constants, obtained by MMX force field calculations ("PC-Model") for a low energy (29.5 kcal) conformation of **(12)** are in good agreement with the spectroscopic data.

All compounds **(4)** - **(12)** were tested for biological activity. Loliolide **(4)**, like isololiolide [13], turned out not to be biologically active at all. Fungicidal effects on *C. cucumerinum* [14] were observed only with acanthospermal B **(8)** and epoxide **(12)** at concentrations of 500 ppm. However, all sesquiterpene

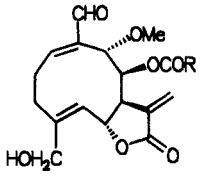
lactones **(5)** - **(12)** showed bactericidal activity against *Bacillus subtilis* (+) and *Pseudomonas stutzeri* (−) with doses of 10 μg and 25 μg, respectively.



5a R = i.–Pr

5b R = CH(Me)CH_2Me

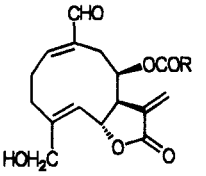
5c R = C(Me)=CH



6a R = CH(Me)CH_2Me

6b R = i.–Pr

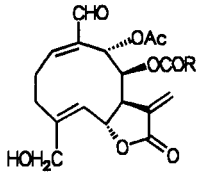
6c R = C(Me)=CH



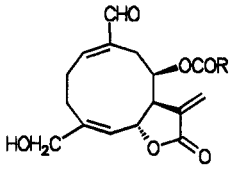
7a R = CH(Me)CH_2Me

7b R = i.–Val

7c R = i.–Pr



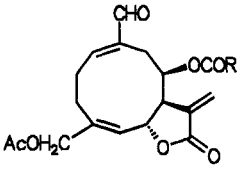
8 R = CH(Me)CH_2Me



9a R = CH(Me)CH_2Me

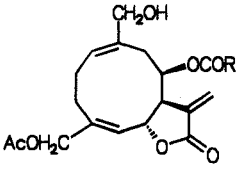
9b R = i.–Pr

9c R = C(Me)=CH

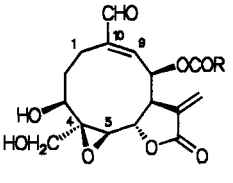


10a R = CH(Me)CH_2Me

10b R = i.–Pr



11 R = CH(Me)CH_2Me



12 R = CH(Me)CH_2Me

TABLE 1. Antifeedant activity of compounds **(5)** - **(12)** towards *E. varivestis*

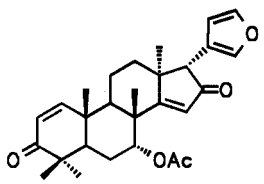
Compound	EC_{50} [ppm]	EC_{100} [ppm]	Compound	EC_{50} [ppm]	EC_{100} [ppm]
(5a)	105	225	**(8)**	105	210
(5b)	105	225	**(9a)**	500	900
(5c)	115	300	**(9b)**	500	950
(6a)	115	300	**(9c)**	600	1050
(6b)	200	400	**(10a)**	900	2000
(6c)	200	400	**(10b)**	900	2000
(7a)	95	200	**(11)**	300	900
(7b)	95	200	**(12)**	115	225
(7c)	115	300			

Higher activities were observed in the dual choice antifeedant tests [1] with *E. varivestis*. The results are listed in table 1. There are big differences observed regarding the antifeedant activity of the 4,5-*Z*-germacranolides (melampolides) as compared to the 4,5-*E* isomers. The melampolides **(5)** - **(8)** and epoxide **(12)** show relatively good feeding inhibition, whereas with the 4,5-*E* germacranolides **(9)** - **(11)** rather high concentrations are needed. From these results it is obvious that the conjugated α-*exo*-methylene group of the lactone ring, reported to be responsible for the biological activity of sesquiterpene lactones in general, is not alone the decisive factor responsible for the antifeedant activity. The stereochemistry at C-4/C-5 and other structural features play also an important role.

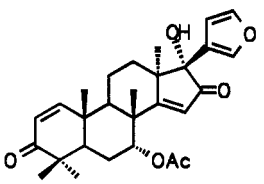
Azadirachta indica seed cake (neem cake) was extracted with water, methanol, and ethyl acetate, and the extracts tested for nematicidal activity. The results are listed in table 2.

TABLE 2. Nematicidal activity of crude neem cake extracts

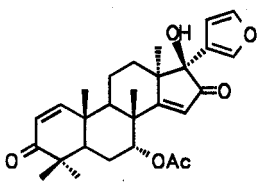
Extraction solvent	Concentration [ppm]	% Abbott
Water	500	95
	100	35
Methanol	500	90
	100	85
Ethyl acetate	500	90
	100	75



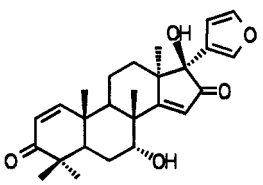
13



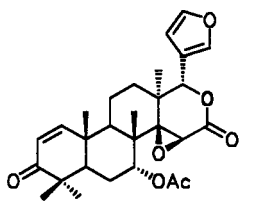
14



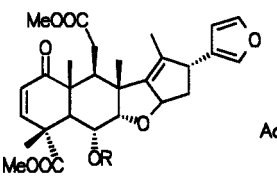
15



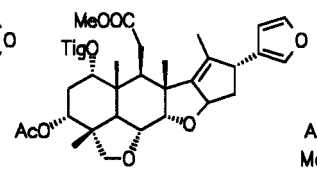
16



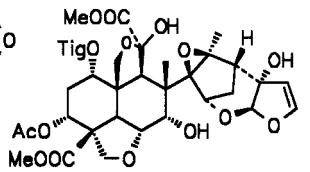
17



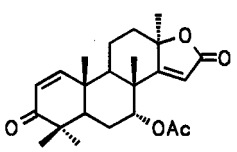
18a/b R = Ac, H



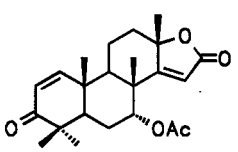
19



20



21



22

Chromatography of the methanol extract yielded several biologically active fractions (70-95 % Abbott) which subsequently were further separated by semipreparative HPLC to give 8 known tetranortriterpenoids **(13)** - **(20)**, and 2 new enneanortriterpenoids, 13α-nimolactone **(21)** and 13β-nimolactone **(22)** [15]. The structures of compounds **(13)** - **(20)** were established by comparison with authentic samples, structures **(21)** and **(22)** on the basis of the ^{1}H and ^{13}C nmr and mass spectra.

The pure compounds **(13)** - **(22)** were tested for nematicidal activity against *M. incognita*. The results are listed in table 3. None of the compounds turned out to be as active as the crude extracts. In order to check for synergistic effects artificial mixtures were prepared, one of them (mixture a) consisting of equal amounts of compounds **(13)**, **(14)**, and **(17)** - **(19)**, the second one (mixture b) representing the original composition of the main components **(13)** - **(22)** as found by HPLC analysis. As can be seen

from table 3, mixture (a) is as active as the components it is consisting of, but mixture (b) is as active as the crude methanol extract. Obviously strong synergistic effects are operating in connection with the nematicidal activity reported earlier on neem cake extracts [5], [6].

TABLE 3. Nematicidal activity in water solution of neem cake constituents

Compound	Concentration [ppm]	% Abbott
Azadiradione **(13)**	100	45
17α-Hydroxyazadiradione **(14)**	100	50
17β-Hydroxyazadiradione **(15)**	100	75
7-Deacetyl-17β-hydroxy-azadiradione **(16)**	100	25
Gedunin **(17)**	100	40
Nimbin + Deacetylnimbin **(18a,b)** (1:1 mixture)	100	30
Salannin **(19)**	100	45
Azadirachtin **(20)**	100	10
13α-Nimolactone **(21)**	100	60
13β-Nimolactone **(22)**	100	50
Mixture (a)	100	50
Mixture (b)	100	85
Crude methanol extract	100	85

Mixture (a):

20 % Azadiradion **(13)**
20 % 17α-Hydroxyazadiradion **(14)**
20 % Gedunin **(17)**
20 % Nimbin/Deacetylnimbin **(18a/b)**
20 % Salannin **(19)**

Mixture (b):

10 % Azadiradione**(13)**
25 % 17α-Hydroxyazadiradione**(14)**
25 % 17β-Hydroxyazadiradione **(15)**
10 % 7-Deacetyl-17β-hydroxyazadiradione **(16)**
5 % Gedunin **(17)**
10 % Nimbin/Deacetylnimbin **(18a/b)**
2.5% Salannin **(19)**
2.5% Azadirachtin **(20)**
2.5% α-Nimolactone **(21)**
2.5% β-Nimolactone **(22)**

The nimolactones **(21)** and **(22)** were active against *Bacillus subtilis* (+) in doses of 10 μg, but not active against *Pseudomonas stutzeri* (−) in doses >200 μg. Moderate feeding inhibition to *E. varivestis* was observed with **(21)** and **(22)** at EC_{50} values of 80 ppm.

References

1. M. Schwinger, B. Ehhammer, and W. Kraus, in H. Schmutterer and K. R. S. Ascher (eds.), *Natural Pesticides from the Neem Tree (Azadirachta indica A. Juss) and Other Tropical Plants* (Proc. 2nd Internat. Neem Conf. Rauischholzhausen 1983), p. 181, GTZ Eschborn 1984.
2. J. Polonsky, *Fortschr. Chem. Org. Naturst.* **30,** 101 (1973).
3. V. Leskinen, J. Polonsky, and S. Bhatnagar, *J. Chem. Ecol.* **10,** 1497 (1984).

4. M. Beck, *Diploma Thesis,* University of Hohenheim, 1991.
5. M. A. Siddiqui and M. M. Alam, *Neem Newsletter* **2** (1), 1 (1985).
6. M. M. Alam, in N. S. Randhawa and B. S. Parmar (eds.), *Neem Research and Development,* Soc. Pest. Sci. India, 1993, p. 123.
7. R. P. Borris, G. A. Cordell, and N. R. Farnsworth, *J. Nat. Prod.* **5,** 641 (1980).
8. S. Siddiqui, T. Mahmood, B. S. Siddiqui, and S. Fainzi, *Planta Medica* **54,** 457 (1987).
9. W. Herz and P. S. Kalyanaraman, *J. Org. Chem.* **40,** 3486 (1975).
10. F. Bohlmann, J. Jakupovic, C. Zdero, R. M. King, and H. Robinson, *Phytochemistry* **18,** 625 (1979); J. Jakupovic, R. N. Baruah, F. Bohlmann, and J. D. Msonthi, *Planta Medica* **52,** 154 (1986).
11. The acanthospermolide derivative **(6b)** has been published recently as a constituent of *Lecocarpus lecocarpoides*: F A. Macias, J. M. G. Molinillo, and N. H. Fischer *Phytochemistry* **32,** 127 (1993).
12. H. D. Fischer, N. H. Fischer, R. W. Franck, and E. J. Olivier, *Fortschr. Chem. Org. Naturst.* **38,** 127 (1979).
13. R. Fleischhacker and W. Kraus, unpublished results.
14. A. L. Homas and A. Fuchs, *J. Chromatogr.* **51,** 327 (1970).
15. Compounds **(21)** and **(22)** have recently been found in the fresh fruit coats of *Azadirachta indica*: B. S. Siddiqui, Ghiasuddin, S. Faizi, and S. Siddiqui, *Phytochemistry* **31,** 4275 (1992).