PERGAMON

PHYTOCHEMISTRY

Phytochemistry 50 (1999) 991–994

Oximes from seeds of *Atalantia ceylanica*

Markus Bacher[a,*], Günter Brader[b], Otmar Hofer[a], Harald Greger[b]

[a]*Institute of Organic Chemistry, University of Vienna, Währingerstrasse 38, 1090 Vienna, Austria*
[b]*Comparative Phytochemistry Department, Institute of Botany, University of Vienna, Rennweg 14, 1030 Vienna, Austria*

Received 1 September 1998

Abstract

The lipophilic seed extract of *Atalantia ceylanica* is characterized by two new oximes, ataloxime A and B, and the known furanocoumarins bergapten, xanthotoxin, heraclenin, oxypeucedanin and imperatorin. The structures of the oximes were elucidated by spectroscopic methods, whereas the furanocoumarins were identified by HPLC-UV and TLC comparisons with authentic samples. The oximes displayed contact toxicity against freshly hatched larvae of the pest insect *Spodoptera littoralis* but did not show antifungal activities against *Cladosporium herbarum*.

Keywords: Atalantia ceylanica; Rutaceae; Seeds; Oximes; Furanocoumarins; Insecticidal activity

1. Introduction

In connection with our screening programme for biologically active compounds of tropical Rutaceae we have investigated the lipophilic seed extract of *Atalantia ceylanica* (Arn.) Oliv. Up to now only limonoids of the propellane-type have been reported for the seeds (Bennett, Hasegawa, & Wong, 1994), whereas the bark, wood and leaves are mainly characterized by acridones and coumarins (Fraser & Lewis, 1973; Ahmad, Shamsuddin, & Zaman, 1984; Bowen & Patel, 1987). In the present paper we report on the isolation and structure elucidation of two new isomeric aldoximes named ataloxime A (**1**) and ataloxime B (**2**). Both compounds have been subsequently tested for their antifungal as well as insecticidal properties.

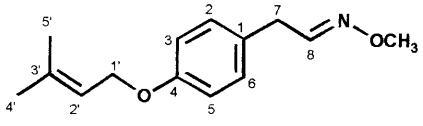
2. Results and discussion

Analytical HPLC analyses of the chloroform phase of the methanolic leaf extract showed two peaks (**1**, **2**) with inconspicuous UV-spectra. Their IR-spectra were nearly identical, with a very strong and prominent absorption at 1510 cm^{-1} and only little differentiation in the fingerprint region. The mass spectra of both compounds were also very similar. Due to the molecular ion peak at $m/z = 233$ the molecular formula of derivatives **1** and **2** was established as $C_{14}H_{19}NO_2$. The fragmentation pattern indicated the presence of a prenyl moiety ($m/z = 69$ and $m/z = 165$ [M-prenyl]) as well as a methoxy group ($m/z = 133$ [M-prenyl–OCH_3]).

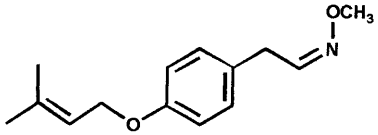
This assumption was confirmed by ^{1}H- and *J*-modulated ^{13}C-NMR measurements. The resonances at δ ca. 3.9 (s, 3H) and δ ca. 62, respectively, are characteristic for methoxy groups, whereas signals at δ ca. 4.5 (d, 2H), 5.5 (tsept, 1H), 1.8 (s, 3H) and 1.75 (s, 3H) are typical for prenyloxy side chains. Moreover, the ^{1}H-NMR spectra of both compounds showed the characteristic pattern of 1,4-disubstituted benzenes with two doublets ($J = 8.5$ Hz, 2H) with nearly the same chemical shift values of approximately δ 7.10 and δ 6.90, respectively. The only differences in the spectra of **1** and **2** were found in the remaining resonances of an AX_2 spin system. Whereas in **1** these protons resonate at δ 7.43 (t, 1H, A-part) and δ 3.45 (d, 2H, X_2-part), in **2** the A-part undergoes a highfield shift of ca 0.7 ppm to δ 6.77 and the X_2-part is shifted to lower frequencies at δ 3.60 ($\Delta\delta = 0.15$ ppm). On the other hand the shift values of the corresponding 'A-carbons' are nearly identical (δ 149.5 for **1** and δ 150.2 for **2**, respectively). However, the shift difference of the two 'X_2-carbons' was $\Delta\delta = 3.64$ ppm, with the carbon of **1** resonating at lower field than that of **2** (δ 35.00 versus δ 31.36). This behaviour of chemical shift changes in both ^{1}H- and ^{13}C-NMR spectra (Karabatsos & Hsi, 1967; Hawkes, Herwig, & Roberts, 1974; Unterhalt & Koehler, 1978; Gordon, Scriba, & Kramer, 1984) and the presence of a methine carbon in the range of δ 150 led to the conclusion, that compounds **1** and **2** are isomeric aldoxime derivatives. Due to the steric compression shift (Bothner-By,

* Corresponding author.

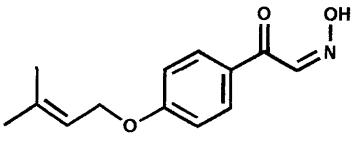
0031-9422/99/$ - see front matter
PII: S0031-9422(98)00638-4



1: Ataloxime A



2: Ataloxime B



3: Citaldoxime

Structure 1.

1960; Buckingham, Schaefer, & Schneider, 1960; Schaefer, Reynolds, & Yonemoto, 1963) carbons in *cis* position to hydroxy or alkyloxy groups (i.e. *cis* or *Z*-aldoximes) resonate at higher fields than *trans* carbons (*trans* or *E*-aldoximes) (Hawkes et al., 1974; Unterhalt & Koehler, 1978). As a consequence, ataloxime A (**1**) is the *trans* and ataloxime B (**2**) the *cis* isomer.

The only remaining question, whether the methoxy or the prenyloxy group is attached to the nitrogen atom, can easily be clarified by careful studies of the ^{1}H and ^{13}C resonances. In both isomers the chemical shift values of the hydrogen atoms in the prenyloxy side chains are nearly identical ($\Delta\delta = 0.01$ ppm) but there was little difference in the shift values of the methoxy groups ($\Delta\delta = 0.07$ ppm). Additionally, the ^{13}C resonances of this substituent in both isomers are at a relatively low field (δ ca 62), whereas methoxy groups bound directly to benzene and having no neighbour in *ortho* position have a shift of approximately δ 55 ppm. Further confirmation that **1** and **2** are *O*-methylaldoximes is given by a detailed analysis of their fragmentation pattern in the mass spectra since the fragment ions $m/z = 165$ and $m/z = 133$ are only consistent with these structures. Loss of the prenyl side chain is accompanied with the formation of a phenolic cation ($m/z = 165$), which in turn undergoes an elimination of methanol forming a cyanide intermediate with $m/z = 133$. Other prominent peaks at $m/z = 107$ and $m/z = 106$ are the result of a separation of either cyanide or prussic acid.

Besides the oximes **1** and **2**, the seed extract of *A. ceylanica* also contains the known furanocoumarins bergapten, imperatorin, xanthotoxin, oxypeucedanin and large amounts of heraclenin, which were identified by HPLC-UV and TLC comparisons with authentic samples. With respect to the already known insecticidal properties of furanocoumarins (Murray, Mendez, & Brown, 1982; Berenbaum, 1991), the growth retarding activity of the crude extract against the polyphagous pest insect *Spodoptera littoralis* may be explained by the presence of these compounds. At a concentration of 1 mg crude extract per g artificial diet, the weight of 5 days old larvae came up only to 17% compared with the control, with 2.5 mg g^{-1} only to 8%. On the other hand, the pure oximes **1** and **2** have no effect on the growth and survival rate of neonate larvae in the chronic feeding bioassay up to a concentration of 0.5 mg g^{-1} (2.2 μmol g^{-1}).

In contrast to the inactivity of the oximes when administered orally, both derivatives (**1**, **2**) caused a pronounced mortality after topical contact in the glass vial bioassay (Nugroho, Schwarz, Wray, & Proksch, 1996). The *cis*-isomer **2** with a LC_{50} of 0.44 μg cm^{-2} (1.9 nmol cm^{-2}) is slightly more active than the *trans*-isomer (LC_{50}: 0.65 μg cm^{-2}; 2.8 nmol cm^{-2}). For comparison, the appropriate LC_{50} value of a pyrethrum extract (containing 25% pyrethrin I and II) was ascertained to be 0.06 μg cm^{-2}. The more active component ataloxime B (**2**) was later also tested for contact toxicity after topical application with elder caterpillars (4th instar). However, in that case the insects were not influenced up to a concentration of 1200 μg g^{-1} fr. wt. of the caterpillar (5 μmol g^{-1} fr. wt.). Here, again the pyrethrum extract caused pronounced mortality with a LC_{50} value of 42 μg g^{-1} fr. wt.

With respect to the already known antifungal activity of citaldoxime (**3**), a stress metabolite of irradiated *Citrus* peel, against *C. cucumerinum* (Dubery, Holzapfel, Kruger, Schabort, & Van Dyk, 1988), the oximes **1** and **2** were also tested for their antifungal activity against *C. herbarum* using bioautography on TLC plates (Greger, Zechner, Hofer, Hadacek, & Wurz, 1993).

However, even at the highest concentration at 200 μg no inhibition of spore germination and mycelial growth could be observed.

Apart from citaldoxime (**3**), which was later also found as a natural product in the roots of different *Citrus* species and cultivars (Ito et al., 1990), oximes represent a rare group of secondary metabolites within the *Rutaceae* family. Citaldoxime (**3**) was supposed to be derived from the glucosinolate pathway and might be related to the aldoxime intermediates resulting from tyrosine as amino acid precursor (Dubery et al., 1988). However, tyrosin derived aldoximes are also involved in the biosynthesis of the cyanogenic glycoside dhurrin, as it was shown

for *Sorghum bicolor* (Halkier & Møller, 1991). In fact, cyanogenic glycosides are already known from the *Rutaceae*, e.g. from the Australian genus *Zieria* (Hegnauer, 1990). It was postulated (Dubery et al., 1988) that in the case of **3** a possible further metabolism of the aldoxime intermediate to glucosinolates (or cyanogenic glycosides) is probably stopped by the introduction of an α-oxo-group. Perhaps the methylation of the isonitroso-group of **1** and **2** also leads to a dead end and prevents further metabolism to more common final products.

3. Experimental

3.1. General

NMR: Bruker AC-250W (250 MHz), $CDCl_3$. MS: Varian MAT-CH7. IR: Perkin-Elmer 16 PC FT-IR. HPLC: UV diode-array detection: 230 nm, column 290 × 4 mm (Spherisorb ODS, 5 μm), mobile phase MeOH (gradient 60–100%) in aq. buffer (*o*-phosphoric acid 0.015 M, tetrabutylammonium hydroxide 0.0015 M, pH = 3), flow rate 1 ml min^{-1}.

3.2. Plant material

Fruits of *A. ceylanica* (Arn.) Oliv. were collected in Minuwangoda, Sri Lanka on February 5th, 1992. Voucher specimens are deposited at the Herbarium of the University of Vienna (WU).

3.3. Extraction and isolation

Fresh seeds (calculated dry weight: 30 g) were coarsely chopped in the field and directly preserved with MeOH. After shipping back to Vienna, the MeOH extract was filtered and concentrated. The $CHCl_3$ fraction from the aqueous solution was evaporated to dryness (1600 mg) under reduced pressure and roughly separated by CC (Merck silica gel 60, 35–70 mesh). Further separation by repeated preparative MPLC with 5% (v/v) EtOAc in petrol (400 × 38 mm column, Merck LiChroprep Si 60, 25–40 μm, UV detection, 254 nm) afforded 28 mg of ataloxime A (**1**) and 40 mg ataloxime B (**2**).

3.4. Bioassays with insects and fungi

Larvae of *Spodoptera littoralis* were from a laboratory colony reared on a bean based artificial diet as it is described in (Srivastava & Proksch, 1991). The chronic feeding bioassays were conducted with freshly hatched larvae ($n = 20$) that were kept on artificial diet spiked with different concentrations of the test compounds (0.25–2.5 μmol g^{-1} fr. wt.). After 5 days (moist chamber, 29°C, darkness) the survival rate and the larval growth of the surviving larvae were monitored in comparison to the control. For contact toxicity the compounds were coated as an even film on the inside of glass vials (inner surface: 75 cm^2) in a dilution series (0.05–1 μmol/dm^2). After evaporation of the carrier (400 μl of Me_2CO) 20 neonate larvae were placed into the vials. Insects were allowed free movement for 3 h in the closed vials. Then diet was added. After 48 h (in a moist chamber, 29°C, darkness) survival was recorded and compared to controls, which were kept in vials treated with Me_2CO only (Nugroho et al., 1996). For a further contact toxicity experiment a known dose of compounds (0.5–100 μg) dissolved in 1 μl Me_2CO was applied to the dorsal side (thorax) of larvae of *S. littoralis* ($n = 5$, fr. wt. 40–70 mg) with a μl syringe. Controls were treated with Me_2CO only. After 48 h, survival was recorded. From the dose–response curves in each experiment (three replicates) LC_{50} values were calculated by probit-log analysis. The Pyrethrum extract containing 25% of pyrethrin I and II was obtained from Fluka. Antifungal tests were performed by bioautography assay on TLC plates. A conidial spore suspension of *Cladosporium herbarum* in malt extract broth was sprayed onto developed TLC-plates. These were kept in a moist chamber for 3 days at room temperature. Antifungal compounds can be detected by white inhibition zones in the growing dark mycelium.

3.5. Ataloxime A {(E)-2-[4-(3-methyl-2-butenyloxy)-phenyl]-ethanaldoxime-methylether} (*1*)

Oil. UV λ^{MeOH} nm: 226, 276, 283 (sh). IR ν^{CCl_4} cm^{-1}: 3030 w, 2962 m, 2934 m, 2818 w, 1612 w, 1582 w, 1510 s, 1464 w, 1442 w, 1382 w, 1298 w, 1238 s, 1176 in, 1086 w, 1038 s, 1006 in, 850 w. ^{1}H NMR ($CDCl_3$): δ 7.43 (t, 1H, $J = 6.6$ Hz, 2′-H), 7.12 (br d, 2H, $J = 8.5$ Hz, 3-H and 5-H), 6.87 (br d, 2H, $J = 8.5$ Hz, 2-H and 6-H), 5.49 (tsept, 1H, $J = 6.6$, ca 1 Hz, 2″-H), 4.49 (d, 2H, $J = 6.6$ Hz, 1″-H), 3.85 (s, 3H, -OMe), 3.45 (d, 2H, $J = 6.6$ Hz, 1′-H), 1.80 (s, 3H, 4″-H), 1.74 (s, 3H, 5″-H). ^{13}C NMR ($CDCl_3$): δ 157.83 (s, 1-C), 149.46 (d, 2′-C) 138.11 (s, 3″-C), 129.72 (d, 3-C and 5-C), 128.18 (s, 4-C), 119.72 (d, 2″-C), 114.93 (d, 2-C and 6-C), 64.82, (t, 1″-C), 61.35 (q, OCH_3), 35.00 (t, 1′-C), 25.79 (q, 4″-C), 18.16 (q, 5″-C). MS (70 eV, 60°C) *m/z* (rel. int.): 233 (3) [M^+, $C_{14}H_{19}NO_2$], 165 (49), 121 (4), 133 (100), 107 (25), 106 (29), 78 (8), 69 (70).

3.6. Ataloxime B {(Z)-2-[4-(3-methyl-2-butenyloxy)-phenyl]-ethanaldoxime-methylether} (*2*)

Oil. UV λ^{MeOH} nm: 222, 276, 283 (sh); IR ν^{CCl_4} cm^{-1}: 3030 w, 2962 w, 2936 in, 2820 w, 1612 w, 1582 w, 1510 s, 1464 w, 1442 w, 1382 w, 1298 w, 1240 s, 1176 m, 1110 w, 1074 w, 1032 s, 1014 m, 874 w, 840 w; ^{1}H NMR ($CDCl_3$): δ 7.10 (br d, 2H, $J = 8.5$ Hz, 3-H and 5-H), 6.86 (br d, 2H, $J = 8.5$ Hz, 2-H and 6-H), 6.77 (t, 1H, $J = 5.5$ Hz, 2′-H), 5.48 (tsept, 1H, $J = 6.6$, ca 1 Hz, 2″-H), 4.48 (d, 2 H,

$J = 6.6$ Hz, 1″-H), 3.92 (s, 3H, -OMe), 3.60 (d, 2H, $J = 5.5$ Hz, 1′-H), 1.79 (s, 3H, 4″-H), 1.73 (s, 3H, 5″-H). ^{13}C NMR ($CDCl_3$): δ 157.65 (s, 1-C), 150.15 (d, 2′-C), 138.11 (s, 3″-C), 129.62 (d, 3-C and 5-C), 128.75 (s, 4-C), 119.71 (d, 2″-C), 114.93 (d, 2-C and 6-C), 64.82, (t, 1″-C), 61.68 (q, OCH_3), 31.33 (t, 1′-C), 25.74 (q, 4″-C), 18.12 (q, 5″-C). MS (70 eV, 60°C) *m/z* (rel. int.): 233 (10) [M^+, $C_{14}H_{19}NO_2$], 165 (37), 133 (100), 107 (20), 106 (33), 78 (12), 69 (74).

Acknowledgements

The present investigation was supported by the Austrian National Committee for the Intergovernmental Programme 'Man and Biosphere'. We are also indebted to the late Professor S. Balasubramaniam, University of Peradeniya, Sri Lanka for his help during plant collection and identification.

References

Ahmad, J., Shamsuddin, K. M., & Zaman, A. (1984). *Phytochemistry*, *23*, 2098.

Bennett, R. D., Hasegawa, S., & Wong, R. Y. (1994). *Phytochemistry*, *36*, 163.

Berenbaum, M. R. (1991). In: G. A. Rosenthal & M. R. Berenbaum (Eds.), *Herbivores. Their interactions with secondary plant metabolites* (pp. 221–249). San Diego: Academic Press.

Bothner-By, A. A. (1960). *J. Mol. Spectrosc.*, *5*, 52.

Bowen, I. H., & Patel, V. N. (1987). *Planta Med.*, *53*, 73.

Buckingham, A. D., Schaefer, T., & Schneider, W. G. (1960). *J. Chem. Phys.*, *32*, 1227.

Dubery, I. A., Hozapfel, C. W., Kruger, G. J., Schabort, J. C., & Van Dyk, M. (1988). *Phytochemistry*, *27*, 2769.

Fraser, A. W., & Lewis, J. R. (1973). *J. Chem. Soc. Perkin Trans.*, *1*, 1173.

Gordon, M. S., Scriba, S. A., & Kramer, J. G. (1984). *J. Org. Chem.*, *49*, 97.

Greger, H., Zechner, G., Hofer, O., Hadacek, F., & Wurz, G. (1993). *Phytochemistry*, *34*, 175.

Halkier, B. A., & Møller, B. L. (1991). *Plant Physiol.*, *96*, 10.

Hawkes, G. E., Herwig, K., & Roberts, J. D. (1974). *J. Org. Chem.*, *39*, 1017.

Hegnauer, R. (1990). *Chemotaxonomie der Pflanzen* (Vol. 9, p. 439). Basel: Birkhauser Verlag.

Ito, C., Tanahashi, S., Tani, Y., Ju-Ichi, M., Omura, M., & Furukawa, H. (1990). *Chem. Pharm. Bull.*, *38*, 2586.

Karabatsos, G. J., & Hsi, N. (1967). *Tetrahedron*, *23*, 1079.

Murray, R. D. H., Mendez, J., & Brown, S. A. (1982) *Naturally occurring coumarins. Occurrence, chemistry, biochemistry*. Chichester: John Wiley and Sons.

Nugroho, B. W., Schwarz, B., Wray, V., & Proksch, P. (1996). *Phytochemistry*, *41*, 129.

Schaefer, T., Reynolds, W. F., & Yonemoto, T. (1963). *Can. J. Chem.*, *41*, 2969.

Srivastava, R. P., & Proksch, P. (1991). *Entomol. Gener.*, *15*, 265.

Unterhalt, B., & Koehler, H. (1978). *Arch. Pharm. (Weinheim)*, *311*, 366.