Pergamon 0031-9422(95)00559-S Phytochemistry, Vol. 41, No. 1, pp. 297–300, 1996
Elsevier Science Ltd
Printed in Great Britain.
0031-9422/96 $15.00 + 0.00

# PEROXIDES AND OTHER CONSTITUENTS FROM *HETEROTHALAMUS ALIENUS*

GERHARD RÜCKER,* ELOIR SCHENKEL,† DETLEF MANNS, MIRIAM FALKENBERG† and ANNE MAREK

Pharmazeutisches Institut der Universität Bonn, Kreuzbergweg 26, 53115 Bonn, Germany; †Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

(*Received* 22 *May* 1995)

**Key Word Index**—*Heterothalamus alienus*; Asteraceae; leaves; docosanoic cinnamate; nerolhydroperoxide; dihydrofuran derivative.

**Abstract**—Three new compounds, docosanoic cinnamate, 7-hydroperoxy-5,6-*E*-dehydro-6,7-dihydronerol and 2-(3-methylbut-3-enyl)-3-methyl-2,5-dihydrofuran were isolated from the leaves of *Heterothalamus alienus* and their structures determined by spectroscopical methods.

## INTRODUCTION

*Heterothalamus alienus* grows as a shrub in the south of Brazil, northern Argentina and Uruguay [1, 2]. In Brazilian folk medicine, a decoction and a powder of the leaves are used internally and externally against fever and as a stimulant [3]. Information about its chemical composition, however, has remained up to now rather poor. Recently, two *ent*-labdaneglycosides have been reported [4].

Previous work [1, 2] pointed to the existence of two morphologically distinct plant populations, both showing the fundamental botanical characteristics of *H. alienus* [5]. The populations were collected at the same place, population A with shrubs up to 1 m high and larger leaves (1–2 cm length) and population B with shrubs up to 2 m high and smaller leaves ( ~ 1 cm length). The populations blossom at different times. Chemical analyses displayed clear differences in the composition of the volatile oils and in that of the main flavonoid components [1, 2, 6]. Herein, we report the structural determination of the isolated compounds of the methylene chloride extracts of both populations.

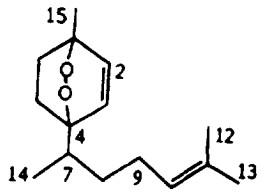
## RESULTS AND DISCUSSION

Chromatographic fractionation [6] of the methylene chloride extract prepared from population A afforded the known substances bisabolene-1,4-endoperoxide (**1**), neryl acetate [7], α- [8] and β-eudesmol [9] (as a mixture), pinocembrin [10, 11] and 2',6'-dihydroxy-4'-methoxy-dihydrochalcone [12]; compound **1** has been isolated earlier from *Rudbeckia laciniata* [13], *Ligularia speciosa* [14], *Chamaemelum fuscatum* [15], *Senecio desfontanei* [16], *Heterothalamus psiadioides* [6, 17], *Eupatorium rufescens* (K. Heiden, unpublished data) and *Senecio selloi* (B. Heinzmann, unpublished data). It shows a significant antimalarial activity *in vitro* against *Plasmodium falciparum* [6, 17]. From population B [1, 2], we isolated the known flavonoids 5,4'-dihydroxy-7-methoxyflavanone (sakuranetin) [18] and 5,4'-dihydroxy-7,3'-dimethoxyflavonol (7,3'-*O*-dimethyl-dihydroquercetin) [19], which were identified by comparison with spectroscopical data given in literature. From population A, the new compounds, docosanoic cinnamate (**2**), 7-hydroperoxy-5,6-*E*-dehydro-6,7-dihydronerol (**3**) and 2-(3'-methylbut-3'-enyl)-3-methyl-2,5-dihydrofuran (**4**) were isolated and identified [6].

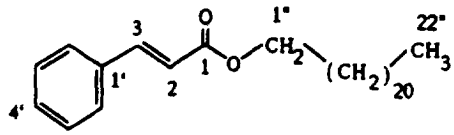
Compound **2** exhibited a $[M]^+$ at *m/z* 456.3952 (calculated for $C_{31}H_{52}O_2$: 456.3967) by HR-EI mass spectrometry. The IR spectrum showed strong bands for ester at 1715 (C=O) and 1170 (C–O) $cm^{-1}$, as well as for a monosubstituted benzene ring at 1640 and 690 $cm^{-1}$. The $^1H$ NMR spectrum showed one triplet for a methyl group (H-22'', $\delta$0.88), two signals for five aromatic protons (H-2', H-4', H-6', $\delta$7.38 and H-3', H-5', $\delta$7.53) and two doublets for an AB-system, indicating the *E*-configurated olefinic protons (H-2, $\delta$6.44; H-3, $\delta$7.68; $J_{2,3}$ = 16 Hz) of a cinnamic ester. Comparison of the NMR data with those for eicosanyl-4'-hydroxy-*trans*-cinnamate [20], as well as for aliphatic straight-chain hydrocarbons resulted in structure **2**.

Fractionation of the petrol-soluble portion of the methylene chloride extract yielded compounds **3** and **4**. Compound **3**, an unstable colourless oil, reacted with peroxide reagent [21] on TLC, indicating that the signal at *m/z* 154 in the FAB-mass spectrum can probably be assigned to $[M - O_2]^+$. In this case, the molecular formula would be $C_{10}H_{18}O_3$. The base peak at *m/z* 135 obviously results from subsequent cleavage of OOH and $H_2O$. An OOH-group and a hydroxyl group were both observed in the $^1H$ NMR spectrum at $\delta$7.8 and $\delta$1.64, respectively (Table 1). Comparison of the $^1H$ NMR data

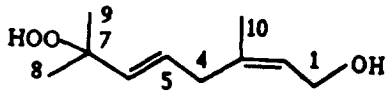
*Author to whom correspondence should be addressed.



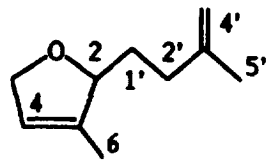

1




2




3




4

Table 1. $^1$H NMR (300 MHz, $CDCl_3$) spectral data and $^{13}$C NMR (75 MHz) spectral data of compound **3**

| H | $\delta$ | Multiplicity | $J$ [Hz] | C | $\delta$ | APT* |
|---|---|---|---|---|---|---|
| 1 | 4.59 | *dq* | 7.0; 1.0 | 1 | 61.1 | $CH_2$ |
| 2 | 5.41 | *tqdd* | 7.0; 1.5 | 2 | 120.1 | CH |
| | | | < 1; < 1 | | | |
| | | | | 3 | 140.0 | C |
| 4 | 2.85 | *d* | 5 | 4 | 35.2 | $CH_2$ |
| 5 | 5.65 | *dd* | 16; 5 | 5 | 127.9 | CH |
| 6 | 5.57 | *d* | 16 | 6 | 134.8 | CH |
| | | | | 7 | 81.9 | C |
| 8 | 1.32 | *s* | | 8 | 24.2 | $CH_3$ |
| 9 | 1.32 | *s* | | 9 | 24.2 | $CH_3$ |
| 10 | 1.75 | *dd* | 1.5; 1.0 | 10 | 23.8 | $CH_3$ |
| OH | 1.64 | *s* | | | | |
| OH | 7.8 | *s* | | | | |

*Theoretical $C/CH_2$ and $CH/CH_3$.

Table 2. $^1$H NMR spectral data of compound **4** (300 MHz, $CDCl_3$)

| H | $\delta$ | Multiplicity | $J$ [Hz] | H, H-COSY correlates with |
|---|---|---|---|---|
| 2 | 4.01 | *dd* | 7.5; 5.0 | H-1′ |
| 4 | 5.37 | *tqd* | 7.5; 1.0; 1.0 | H-6, H-5 |
| 5a | 4.62 | *ddq* | 12.5; 7.5; 1.0 | H-6, H-4 |
| 5b | 4.56 | *ddq* | 12.5; 7.5; 1.0 | H-6, H-4 |
| 6 | 1.77 | *dd* | 2.5; 1.0 | H-5, H-4 |
| 1′ | 1.64 | *m* | (8.0; 7.5; 7.0; 5.0) | H-2′, H-2 |
| 2′ | 2.17 | *m* | (6.5; 7.0; 8.0) | H-1′ |
| 4′a | 4.85 | *qdd* | 1.5; 1.5; 1.0 | H-5′ |
| 4′b | 4.95 | *qtd* | 1.0; 1.0; 1.0 | H-5′ |
| 5′ | 1.73 | *dd* | 1.5; 1.0 | H-4′ |

with those of 7-hydroperoxy-5,6-*E*-dehydro-6,7-dihydroneryl acetate and the corresponding dihydrogeranylacetate [22], both isolated from *Mutisia spinosa*), allowed the assignment of 7-hydroperoxy-5,6-*E*-dehydro-6,7-dihydronerol (**3**). The data differ mainly in the missing signal for the acetyl group and in the shifts of the signals for protons H-1 ($\delta$4.59, literature: 5.15 and 5.14) and H-2 ($\delta$5.41, literature: 5.98). The $^{13}$C APT spectrum (Table 1), not reported previously [22] for the above mentioned neryl- and geranyl derivatives, is in accordance with this structure. The $\Delta$ 2,3-double bond ought to be *Z*-configurated, because the resonance for the methyl group at C-10 appears with a characteristic downfield shift ($\delta$23.8) [23], like that in nerol [7].

Table 3. $^{13}$C NMR spectral data of compound **4** (75 MHz, $CDCl_3$)

| C | $\delta$ | APT* | HETCOR correlates with |
|---|---|---|---|
| 2 | 75.0 | CH | H-2 |
| 3 | 142.2 | C | |
| 4 | 119.5 | CH | H-4 |
| 5 | 61.1 | $CH_2$ | H-5 |
| 6 | 23.4 | $CH_3$ | H-6 |
| 1′ | 33.2 | $CH_2$ | H-1′ |
| 2′ | 28.1 | $CH_2$ | H-2′ |
| 3′ | 147.2 | C | |
| 4′ | 110.9 | $CH_2$ | H-4a, H-4b |
| 5′ | 17.8 | $CH_3$ | H-5′ |

*Theoretical C/$CH_2$ and CH/$CH_3$.

The HR EI mass spectrum of compound **4** showed a $[M]^+$ at *m/z* 152.1208 (calculated: 152.1201) corresponding to a molecular formula of $C_{10}H_{16}O$, with three double bond equivalents. The $^{13}$C APT spectrum (Table 3) showed signals for two methyl groups (C-5′, $\delta$17.8; C-6, $\delta$23.4), both linked to olefinic carbon atoms, and two methylene groups (C-1′, $\delta$33.2; C-2′, $\delta$28.1). Two signals for oxygen-substituted carbon atoms, one for a methylene group at $\delta$61.1 (C-5) and one for a methine group at $\delta$75.0 (C-2), as well as four signals for olefinic carbon atoms (C-4, $\delta$119.5; C-3, $\delta$142.2; C-3′, $\delta$147.5; C-4′, $\delta$110.9) could also be observed at lower field. In the $^1$H NMR spectrum (Table 2), the oxygen-substituted methylene group (H-5) gave a *ddq* at $\delta$4.6, showing besides geminal couplings ($J_{gem}$ = 12.5 Hz), couplings to the methine group H-4 ($J_{5,4}$ = 7.5 Hz) and homoallylic couplings to the methyl group H-6 ($J_{5,6}$ = 1.0 Hz). The signal of the oxygen-substituted methine group at H-2 ($\delta$4.01) appeared as a double doublet, showing different couplings to the two protons of the methylene group H-1′ at $\delta$1.64 ($J_{2,1'a}$ = 7.5 Hz; $J_{2,1'b}$ = 5 Hz). The four olefinic carbon atoms correspond to two double bond equivalents, leaving one left for a ring. Because there was no signal for a hydroxyl group in the $^1$H NMR spectrum and the molecular formula contains only one oxygen atom, the only possibility left is a five-membered oxygen-containing ring. This was confirmed by the characteristic shifts of the carbon atoms C-2 and C-5 (Table 3). Furthermore, signals for an isoprenyl side-chain were observed, which ought to be attached to the furan ring at C-2, because the methine proton H-2 appeared as a double doublet in the $^1$H NMR spectrum. The multiplicity of the methylene protons of H-2′ is explained by their proximity to the methylene protons, H-1′, and the isopropenylic methylene protons, H-4′. These are magnetically not equivalent and give signals at $\delta$4.85 and $\delta$4.95, both with further couplings to the methyl group at H-5′. Through homo- and heteronuclear COSY experiments (Tables 2, 3), all signals could be assigned and result in structure **4**, 2-(3′-methylbut-3′-enyl)-3-methyl-2,5-dihydrofuran. An isomeric compound, 2-(3′-methylbut-2′-enyl)-3-methyl-2,5-dihydrofuran, has already been isolated from *Artemisia pallens* [24], but the spectral data (no solvent or technical data given), especially for the carbon atoms C-2 (literature $\delta$64.0) and C-4 (literature $\delta$129.3), differ markedly, compared to those of **4**.

## EXPERIMENTAL

Mp: uncorr. IR: $CCl_4$. UV: MeOH. MS: 70 eV. NMR (300 MHz for $^1$H, 75 MHz for $^{13}$C): $CDCl_3$. TLC was carried out on silica gel plates and peroxides were detected with peroxide reagent [21].

*Plant material, population A* [6]. Leaves of *H. alienus* (Spreng.) O. Kuntze were collected in October 1990 in Santana da Boa Vista, Rio Grande do Sul, Brazil. A voucher specimen (No. Sobral 6596) is preserved at the Herbário do Instituto de Biociências (ICN) at the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

*Extraction and isolation.* Leaves of population A (800 g) were chopped, soaked with $CH_2Cl_2$, macerated at room temp. for 8 days, filtered and concd by vacuum evapn. The crude extract obtained (14.5 g) was suspended in 90% MeOH and extracted 3× with petrol. After solvent evapn, 12 g of extract were obtained from the MeOH phase and 2.5 g from the petrol phase.

The MeOH phase was fractionated by flash CC on silica gel (0.063–0.2 mm Merck), eluting gradually with *n*-hexane–$Me_2CO$ 19:1 and 9:1, $CH_2Cl_2$ and MeOH, to give 11 frs (Me I–Me XI). Fr. Me IV (1.03 g) was submitted to repeated CC (silica gel, *n*-hexane–$Me_2CO$, 49:1), yielding 32 mg of **1**, 180 mg of neryl acetate and 49 mg of **2**. Fractionation of Me V (370 mg) resulted in a mixt. of $\alpha$- and $\beta$-eudesmol (48 mg), while CC of frs Me VIII (1.49 g) and Me IX (200 mg) gave 22 mg of pinocembrin and 33 mg of 2′,6′-dihydroxy-4′-methoxy-dihydrochalcone, respectively.

The petrol phase was fractionated by flash CC over silica gel, eluting with petrol–$Me_2CO$, 49:1 and 9:1, petrol–EtOAc, 4:1, $CH_2Cl_2$ and EtOH, to give 16 frs (Pe I–Pe XVI). Fr. Pe XVI (1.24 g) was fractionated by CC (silica gel, toluene–EtOAc, 19:1 and 7:3, $CH_2Cl_2$, EtOH) to give 9 frs (Pe XVI-1–Pe XVI-9). Fr. Pe XVI-6 (29 mg) was submitted to CC (silica gel, petrol–EtOAc, 17:3) and gave 6.8 mg of **3**. Fr. Pe XVI-8 (170 mg) was subjected to repeated CC (silica gel, petrol–EtOAc, 87:13, petrol–$Me_2CO$, 9:1) and afforded 10 mg of **4**.

*Docosanoic cinnamate* (**2**). Fine needles, mp 54–56°. $R_f$ 0.35 (*n*-hexane–$Et_2O$, 19:1). UV: $\lambda_{max}$ nm = 270 and 302. IR: $\nu_{max}$ cm$^{-1}$: 2915 *s*, 2860 *s*, 1715 *s*, 1640 *m*, 1470 *m*, 1310 *m*, 1170 *s*. EIMS *m/z* (rel. int.) 456 $[M]^+$ (29), 307 $[C_{22}H_{43}]^+$ (10), 306 $[C_{22}H_{42}]^+$ (49), 189 $[C_{12}H_{13}O_2]^+$ (12), 150 $[C_9H_{10}O_2]^+$ (11), 149 $[C_9H_9O_2]^+$ (67), 148 $[C_9H_8O_2]^+$ (100), 147 $[C_9H_7O_2]^+$ (15), 131 $[C_9H_7O]^+$ (46). $^1$H NMR: $\delta$0.88 (3H, *t*, $J_{22'',21''}$ = 7 Hz, Me-22″), $\delta$1.20, 1.4 (38H, *m*, H-3″ to H-21″), $\delta$1.70 (2H, *tt*, $J_{1''} = J_{3''}$ = 7 Hz, H-2″), $\delta$4.2 (2H, *t*, $J_{1'',2''}$ = 7 Hz, H-1″), $\delta$6.44 (1H, *d*, $J_{2,3}$ = 16 Hz, H-2), $\delta$7.38 (3H, *m*, H-2′, H-4′, H-6′), $\delta$7.53 (2H, *m*, H-3′, H-5′), $\delta$7.68 (1H, *d*, $J_{3,2}$ = 16 Hz, H-3). $^{13}$C NMR: $\delta$14.2 ($CH_3$, C-22″), $\delta$22.8 ($CH_2$, C-21″), $\delta$26.1 ($CH_2$, C-19″*), $\delta$28.8 ($CH_2$, C-17″*),

$\delta$29.36–29.77 ($CH_2$, C-3″ to C-16″, C-18″, C-20″), $\delta$31.9 ($CH_2$, C-2″), $\delta$64.8 ($CH_2$, C-1″), $\delta$118.2 (CH, C-2), $\delta$127.9 (2 × CH, C-2′, C-6′), $\delta$128.8 (2 × CH, C-3′, C-5′), $\delta$130.1 (CH, C-4′), $\delta$134.4 (C, C-1′), $\delta$144.4 (CH, C-3), $\delta$166.9 (C, C-1); # assignments may be interchanged.

*7-Hydroperoxy-5,6-E-dehydro-6,7-dihydronerol* (**3**). Oil. $R_f$ 0.18 (petrol–EtOAc, 17:3). FAB-MS (70 eV, mNBA + NaOAc) *m/z* (rel. int.): 154 ($[M - O_2]^+$), 135 (100), *m/z* 109. $^1$H and $^{13}$C NMR: Table 1.

*2-(3′-Methylbut-3′-enyl)-3-methyl-2,5-dihydrofuran* (**4**). Oil. $R_f$ = 0.17 (petrol–$Me_2CO$, 9:1); MS *m/z* (rel. int.) 152 $[M]^+$ (19), 137 (29), 134 $[C_{10}H_{14}]^+$ (49), 126 $[C_7H_{10}O_2]^+$ (28), 119 $[C_9H_{11}]^+$ (49), 110 $[C_7H_{10}O]^+$ (22), 97 $[C_6H_9O]^+$ (32), 93.07 $[C_7H_9]^+$ (37), 91 $[C_7H_7]^+$ (23), 84 $[C_5H_8O]^+$ (100), 82 $[C_6H_{10}]^+$ (60), 79 $[C_6H_7]^+$ (37), 71 $[C_4H_7O]^+$ (57), 69.07 $[C_5H_9]^+$ (49), 69.03 $[C_4H_5O]^+$ (45), 68 $[C_5H_8]^+$ (65), 67 $[C_5H_7]^+$ (94). $^1$H NMR: Table 2. $^{13}$C NMR: Table 3.

*Plant material, population B* [1, 2]. Leaves of *H. alienus* were collected in May 1990 in Santana da Boa Vista, Rio Grande do Sul, Brazil. A voucher specimen (No. Sobral 7517) is preserved at the Herbário do Instituto de Biociências (ICN) at the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

*Extraction and isolation.* Leaves of population B (855 g) were treated in the same way as described for population A, giving 7.2 g of crude extract. This was processed in the same way as described above, yielding 4 g of extract from the MeOH phase and 3.2 g of extract from the petrol phase. The MeOH phase was fractionated by flash CC on silica gel eluting gradually with petrol–EtOAc from 10 to 100% to give 65 frs (EB 1–EB 65). Frs EB 23–25 (722 mg) were suspended in EtOAc and the ppt. submitted to recrystallisation from $CHCl_3$ and EtOAc, yielding 109 mg of sakuranetin; $R_f$ 0.46 (petrol–EtOAc, 3:2). Frs EB 37–39 (744 mg) afforded 50 mg of (2*R*,3*R*)-7,3′-dimethyldihydroquercetin after repeated recrystallisation from petrol and EtOAc; $R_f$ 0.26 (petrol–EtOAc, 3:2).

*Acknowledgements*—We are grateful to Nélson Matzenbacher and Marcos E. G. Sobral for locating and helping to collect the plant material and for the identification of both populations of *H. alienus*.

## REFERENCES

1. Falkenberg, M. B. (1991) Dissertação de Mestre em Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.
2. Falkenberg, M. B., Ribeiro, D. R. and Schenkel, E. P. (1991) *Caderno de Farmácia (Supl.)* **7**, 12.
3. Pio Corrêa, M. (1984) in *Diccionario das Plantas Úteis do Brasil e das Exóticas Cultivadas* **I**, p. 55, Ministerio da Agricultura, Instituto Brasileiro de Desenvolvimento Florestal.
4. Saad, J. R. and Giordano, O. S. (1993) *J. Nat. Prod.* **34**, 1944.
5. Barroso, G. M. (1973) Compositae – Sub-tribo Baccharidinae Hoffmann. Estudo das espécies ocorrentes no Brasil. Campinas, Universidade Estadual de Campinas, *Tese de Doutorado em Ciências.*
6. Marek, A. (1994) *Dissertation*, Pharmazeutisches Institut, Universität Bonn.
7. Wehrli, F. W. and Nishida, T. (1979) *Progress in the Chemistry of Organic Natural Products* **34**, 24.
8. Humber, D. C. and Pinder, A. R. (1966) *Tetrahedron Letters* **41**, 4985.
9. Paulini, G. (1989) *Dissertation*, Pharmazeutisches Institut, Universität Bonn.
10. Lasswell, W. L. Jr. and Hufford, C. D. (1977) *J. Org. Chem.* **42**, 1295.
11. Harborne, J. B. and Mabry, T. J. (1982) in *The Flavonoids – Advances in Research*, Chapman and Hall, London.
12. Star, A. E. and Mabry, T. J. (1971) *Phytochemistry* **10**, 2817.
13. Bohlmann, F., Jakupovic, J. and Zdero, C. (1978) *Phytochemistry* **17**, 2034.
14. Bohlmann, F. and Fritz, U. (1980) *Phytochemistry* **19**, 2471.
15. Pascual, J., de Caballero, E., Anaya, J. and Gonzalez, M. S. (1983) *Phytochemistry* **22**, 1757.
16. Metwally, M. A. and Dawidar, A. A. (1986) *Pharmazie* **41**, 522.
17. Rücker, G., Walter, R. D., Manns, D, Mayer, R. (1991) *Planta Med.* **37**, 295.
18. Nagarajan, G. R. and Parmar, V. S. (1977) *Planta Med.* **32**, 50.
19. Timmermann, B. N., Hoffmann, J. P., Jolad, S. D., Bates, R. B. and Siahann, T. J. (1986) *Phytochemistry* **25**, 723.
20. Kisiel, W. and Jakupovic, J. (1995) *Planta Med.* **41**, 87.
21. Rücker, G., Neugebauer, M. and Kiefer, A. (1995) *Pharmazie*, **50**, 601.
22. Zdero, C., Bohlmann, F., King, R. M. and Robinson, H. (1986) *Phytochemistry* **23**, 509.
23. De Haan, J. W. and van de Ven, J. M. (1973) *Org. Magn. Reson.* **3**, 147.
24. Chandra, A., Misra, L. N. and Thakur, R. S. (1987) *Tetrahedron Lett.* **28**, 6377.