Phthalide Derivatives from *Meum athamanticum* Jacq.

Mourad Kaouadji, Anne-Marie Mariotte,

Laboratoire de Pharmacognosie, UER de Pharmacie, Université Scientifique et Médicale de Grenoble, Domaine de La Merci, F-38700 La Tronche

and Henri Reutenauer

Laboratoire de Résonance Magnétique (CGRM), Centre d'Etudes Nucléaires de Grenoble, 85 X, F-38041 Grenoble Cédex

Z. Naturforsch. **39c**, 872–875 (1984); received March 26, 1984

Meum athamanticum, Umbelliferae, Sedanenolide, Ligustilidiol, 6.6′,7.3′a-Diligustilide

A detailed investigation of non polar extracts of *Meum athamanticum* Jacq. rhizome (Umbelliferae) has led to the isolation of three phthalide derivatives. Sedanenolide, 6.6′,7.3′a-diligustilide and ligustilidiol have been identified by their spectral data. Furthermore, occurrence of such compounds seems to suggest assignment of intermediate biogenetic part to (*Z*)-ligustilide, the most accumulated phthalide in umbelliferous plants.

Introduction

Meum athamanticum Jacq., a perennial plant of Central and Western Europe mountains, is a member of Umbelliferae (Apiaceae). Previous chemical investigations of this species resulted in identification of some phthalides in the essential oil fruit, ligustilide, 3-butylidene- and 3-*n*-butyl-phthalide [1] and besides ligustilide and some monoterpenes, a C_{11}-hydrocarbon named viridene, from the essential oil rhizome [2]. In the other hand, the methanolic rhizome extract yielded 1-*trans*-O-caffeoyl- and 1-*trans*-O-feruloyl-quinic acid methyl ester [3]. Further examination of non polar rhizome extracts issued in the isolation of sedanenolide (**1**), (*Z*, *Z*′)-rel-(6*S*, 7*S*)-6.6′,7.3′a-diligustilide (**2**) and (*Z*)-rel-(6*S*, 7*S*)-6,7-dihydroxyligustilide or ligustilidiol (**3**).

Results and Discussion

n-hexane extract of *Meum athamanticum* rhizome contains, besides phthalides of common occurrence in the Apiaceae, two other members sedanenolide (**1**) and (*Z*, *Z*′)-6.6′,7.3′a-diligustilide (**2**) which are identified by their spectral data. Defatted rhizome, when submitted to chloroform, gives a viscous mass from which (*Z*)-ligustilidiol (**3**) is extracted and characterized.

*Sedanenolide (**1**)*

Isolated from a mixture containing (*Z*)-3-butylidene-phthalide, (*Z*)-ligustilide and 3-*n*-butylphthalide identified by ^1H NMR and MS data, compound **1** is purified by repeated CC of silica. Its ^1H NMR spectrum ($CDCl_3$; 250 MHz) exhibites signals relative to two *cis*-ethylenic protons, as observed for ligustilide (**4**), at δ 6.21 ppm – broad d – *J* 10 Hz and δ 5.91 ppm – dt – *J* 10 and 3.5 Hz, corresponding respectively to H-7 and H-6, associated with two deshielded methylene groups appearing as overlapped multiplets centered at δ 2.47 ppm (H-4 and H-5). In contrast with ligustilide ($C_{12}H_{14}O_2$ *m/z* 190), no butylidene chain takes place in compound **1** ($C_{12}H_{16}O_2$ *m/z* 192) but a *n*-butyl one of which the non equivalent H of a methylene group are located at δ 1.88 ppm – m – *J* 14, 7 and 4 Hz and δ 1.56 ppm – m – *J* 14 and 6.5 Hz, besides two isochrone methylenes centered at δ 1.39 ppm – m and one methyl at δ 0.90 ppm – t – 7 Hz. This side chain is linked to the five-membered ring lactone (ν_{max} 1750 cm^{-1}) by a deshielded methine δ 4.93 ppm – dd – *J* 6.5 and 4 Hz (H-3). Consequently, compound **1** is identified with 3-*n*-butyl-4,5-dihydrophthalide whose spectral data are in agreement with those reported for senkyunolide and sedanenolide isolated first, at the same time, from two umbelliferous plants *Cnidium officinale* [4] and *Apium graveolens* [5] respectively.

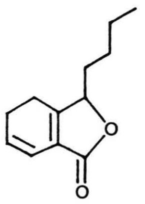
*(Z, Z′)-6.6′,7.3′a-diligustilide (**1**)*

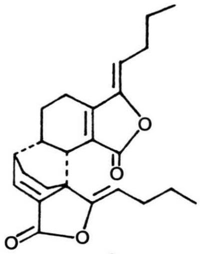
The presence of two butylidene chains in the ^1H NMR spectrum of compound **2**, confirmed by

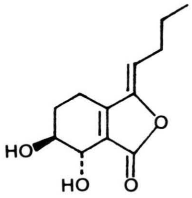
---

Reprint requests to M. Kaouadji.

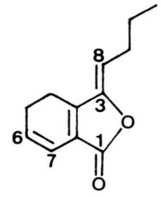
0341-0382/84/0900-0872 $ 01.30/0


1

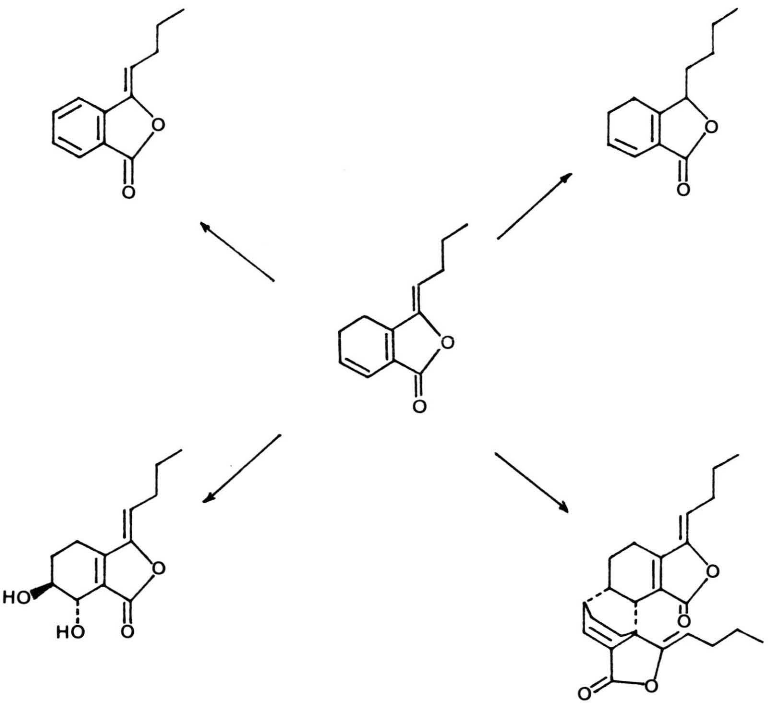

2



3



4



Scheme I

double irradiation experiments, strongly suggests a dimeric structure for this natural product. Effectively, the mass spectrum exhibites the molecular ion at *m/z* 380 corresponding to $C_{24}H_{28}O_4$ (high resolution) and the base peak is observed at *m/z* 190: $C_{12}H_{14}O_2^{+\cdot}$, a fragment ion generated by a *retro*-Diels-Alder reaction from $M^{+\cdot}$. Compound **2** is thus considered a dimer of ligustilide (**4**). As no *cis*-ethylenic protons appear in the ^1H NMR spectrum, dimerisation affecting each of the two ligustilide units is suspected.

Analysis of the ^1H NMR spectrum ($CDCl_3$; 250 MHz) shows four methines at δ 7.36 ppm – d – J 6.5 Hz, δ 3.27 ppm – broad d – J 9 Hz, δ 3.00 ppm – m – J 6.5, 2.5, 2.5 and 2.5 Hz and δ 2.56 ppm – broad ddd – J 9, 6.5 and 2.5 Hz. Each of the two doublets is assigned to one methine obligatory linked to one quaternary C-atom and particulary one ethylenic quaternary C-atom, on the basis of deshielding recorded δ 3.27 and 7.36 ppm respectively, the last δ-value indicating furthermore an ethylenic H. The two next methines δ 3.00 and 2.56 ppm respectively, are adjacent and included between the deshielded ones, leading to a tetrasubstituted bicyclo[2.2.2]octene. This is supported by decoupling experiments giving evidence for four endocyclic methylenes:

- δ 2.06 ppm – m – J 10, 5 and 2 Hz;
  δ 2.02 ppm – dd – J 10 and 4 Hz.
- δ 1.58 ppm – broad dd – J 12 and 6.5 Hz;
  δ ca. 1.40 ppm – m.
- δ 1.95 ppm – m – J 13, 3 and 1.5 Hz;
  δ 1.87 ppm – m – J 13, 4 and 2.5 Hz.

δ 1.56 ppm – broad d – J 12 Hz;
δ 1.33 ppm – m – J 12, 4 and 2.5 Hz,

and indicating that the last two (δ 1.95 and 1.87 ppm, δ 1.56 and 1.33 ppm) belong to the bicyclooctene which is only generated by the 6,7-double bond of the two ligustilide units. From those results, it becomes possible to assign δ 2.56 ppm to H-6, δ 3.27 ppm to H-7, δ 3.00 to H-6′, δ 7.36 ppm to H-7′, to definite the 6.6′-link and to deduce the 7.3′a one.

The presence of two butylidene chains in compound **2** is shown by signals relative to two ethylenic H, four methylene groups and two isochrone methyls in the ^{1}H NMR spectrum. Irradiation experiments permit to put together the groups involved in the same chain as following:

– $CH_3{-}CH_2{-}CH_2(a){-}CH(b){=}\overset{|}{C}{-}$ with CH_3: δ 0.92 ppm – t – J 7 Hz; CH_2: δ 1.45 ppm – m – J 7.5 and 7 Hz; CH_2(a): δ 2.18 ppm – m – J 7.5 and 7.5 Hz; CH(b): δ 5.00 ppm – t – J 7.5 Hz.

– $CH_3{-}CH_2{-}CH_2(a'){-}CH(b'){=}\overset{|}{C}{-}$ with CH_3: δ 0.92 ppm – t – J 7 Hz; CH_2: δ 1.45 ppm – m – J 7.5 and 7 Hz; CH_2(a′): δ 2.30 ppm – m – J 8 and 7.5 Hz; CH(b′): δ 5.08 ppm – t – J 8 Hz.

Each of the two side chain is linked to a five ring lactone appearing in the IR spectrum at 1790 and 1775 cm^{-1} respectively.

Conclusion about the stereochemistry of the butylidene chains can be deduced from the chemical shift values of H-8 and H-8′ (δ 5.08 and 5.00 ppm) relative to Z and Z' forms, more shielded than those found in monomeric compounds as Z-ligustilide (δ 5.23–5.28 ppm) [6, 7] and (Z)-ligustilidiol (δ 5.22–5.25 ppm) [8]. In the other hand, the vicinal coupling occurring between H-6 and H-7 (J 9 Hz) is associated with a diaxial orientation and the relative configuration 6S, 7S is thus assigned to compound **2** which partially leads to (Z)-ligustilide, when submitted to thermolysis.

(Z, Z')-rel-(6S, 7S)-6.6′,7.3′a-diligustilide **2**, isolated first from *Ligusticum wallichii* rhizome [9], has been just described in *Levisticum officinale* rhizome [10] where it is more accumulated than other dimers.

*Ligustilidiol (**3**)*

This other (Z)-ligustilide derivative is recognized by its mass spectrum which exhibites the molecular ion at m/z 224 ($C_{12}H_{16}O_4$ obtained by high resolution MS) and the base peak at m/z 180 arisen from a RDA reaction. The same fragmentation is observed for the di-TMSi derivative (m/z 368) which base peak occurs at m/z 252. The ^{1}H NMR spectrum (Py-D_5; 250 MHz) displays a butylidene chain and a tetrasubstituted cyclohexene ring; the last part of this molecule is identified, by the strong IR band at 1755 cm^{-1}, with a five ring lactone.

Examination of the ^{1}H NMR spectrum of compound **3**, after irradiation experiments, gives evidence for two adjacent methines at δ 5.02 ppm – d – J 3.5 Hz and δ 4.60 ppm – m – J 4.5, 3.5 and 1.5 Hz besides two adjacent methylenes which non equivalent H are recorded at δ 2.79 ppm – m – J 18, 10 and 3.5 Hz, δ 2.45 ppm – m – J 18, 6.5 and 1.5 Hz, δ 2.35 ppm – m – J 13.5, 10, 6.5 and 4.5 Hz, δ 2.17 ppm – m – J 13.5, 1.5 and 1.5 Hz. Each methine group is O-bound as indicated by deshielding (δ4.60 and 5.02 ppm), the last one being linked to a tetrasubstituted double bond carrying one of the methylene groups (δ 2.79 and 2.45 ppm). Those data are in agreement with a 1,2,3,4,-tetrasubstituted cyclohexene ring which insaturation is responsible of the UV absorption (λ_{max} 272 nm) with the carbonyl function and the butylidene chain $CH_3{-}CH_2(a){-}CH_2(b){-}CH{=}\overset{|}{C}{-}$: CH_3: δ 0.85 ppm – t – J 7.5 Hz; CH_2(a): δ 1.38 ppm – m – J 7.5 and 7.5 Hz; CH_2(b): δ2.25 ppm – m – J 7.5 and 7.5 Hz; CH: δ5.24 ppm – t – J 7.5 Hz.

As for 6.6′,7.3′a-diligustilide (**2**), compound **3** occurs in the Z-form with relative configuration 6S, 7S according to couplings recorded between H-6 and H-7 (J 3.5 Hz), H-6 and H-5 (J 4.5 and 1.5 Hz) jointly definiting H-6 equatorial and H-7 axial.

(*Z*)-rel-(6*S*, 7*S*)-6,7-dihydroxyligustilide, named ligustilidiol (**3**) has been first isolated from *Ligusticum wallichii* rhizome [8] and is described here for the second time.

Phthalides and related compounds were first extracted from essential oils of umbelliferous plants and only hydrocarbons were found. Recently, use of solvents in increasing polarity by maceration of the vegetal material has led to the isolation of, besides monomeric hydrocarbons, dimeric ones in the less polar extracts and hydroxylated derivatives in the more polar. This proceeding has permitted enhancement of this group of natural products where, both dimers and ligustilidiol contribute to suggest some relation between all them through ligustilide, as illustrated in Scheme I. One can notice the part played by the 6,7-double bond in (*Z*)-ligustilide (**4**), leading to hydroxy- and dimeric derivatives. On the other hand, this allow to suspect the presence of related compounds in umbelliferous plants, generated from sedanenolide (**1**) for instance, by similar biosynthetic pathways.

Experimental

The general methods are the same as reported in ref. [3]. Extraction of sedanenolide (**1**) and 6.6′,7.3′a-diligustilide (**2**), is effected in using *n*-hexane extract corresponding to 100 g of *Meum athamanticum* dried rhizome. Purification is carried out by repeated CC of silica, with an elution gradient from benzene up to $CHCl_3$ yielding 21 mg of **1** and 12 mg of **2**. The $CHCl_3$ extract is partitioned by repeated polyamide CC with increasing amounts of MeOH in benzene. 7 mg of ligustilidiol (**3**) are obtained after further purification through a Sephadex LH 20 column eluted with MeOH.

*Sedanenolide (**1**):* UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (ε): 275 (3520). IR $\nu_{\max}^{\text{liquid film}}$ $\mathrm{cm^{-1}}$: 2930, 1750, 1650, 1460, 1430, 1340, 1270, 1040, 955, 910 and 840. MS (70 eV) *m/z* (%): 192 ($M^{+\cdot}$; 192.1156; $C_{12}H_{14}O_4 = 192.1150$), 163 (3), 135 (4), 133 (10), 107 (100), 105 (8), 91 (8), 85 (16), 79 (58), 77 (42), 63 (4) and 57 (16). ^1H NMR (see text).

*6.6′,7.3′a-diligustilide (**2**):* UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (ε): 272 (12700). IR $\nu_{\max}^{\text{liquid film}}$ $\mathrm{cm^{-1}}$: 2930, 1790, 1775, 1715, 1640, 1620, 1560, 1465, 1425, 1370, 1265, 1175, 1050, 1025, 975, 955 and 885. MS (70 eV) *m/z* (%): 380 ($M^{+\cdot}$; 4.5; 380.1980; $C_{24}H_{28}O_4$ 380.1987), 190 (100; 190.09977; $C_{12}H_{14}O_2 = 190.0994$), 161 (40), 148 (60), 133 (10), 120 (10), 115 (10), 105 (12), 91 (8), 78 (12), 69 (8) and 55 (38). ^1H NMR (see text).

*Ligustilidiol (**3**):* UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (ε): 272 (8550). IR $\nu_{\max}^{\text{liquid film}}$ $\mathrm{cm^{-1}}$: 3500, 2950, 1755, 1675, 1640, 1580, 1470, 1420, 1380, 1225, 1100, 1040, 970, 800 and 730. MS (70 eV) *m/z* (%): 224 ($M^{+\cdot}$; 98; 224.1030; $C_{12}H_{16}O_4 = 224.1048$), 180 (100; 180.0803; $C_{10}H_{12}O_3 = 180.0786$), 165 (35; 165.0549; $C_9H_9O_3 = 165.0552$), 151 (98; 151.0373; $C_8H_7O_3 = 151.0395$), 138 (40), 123 (28), 95 (32), 77 (20) and 55 (80). ^1H NMR (see text).

*Di-TMSi derivative of **3**:* MS (70 eV) *m/z* (%): 368 ($M^{+\cdot}$; 65), 353 (30), 252 (100), 237 (18), 224 (30) and 147 (28).

[1] E. Stahl and H. Bohrmann, Naturwissenschaften **54,** 118 (1967).
[2] K. H. Kubeczka, V. Formacek, and M. Grünsfelder, in Natural Products of Higher Plants, Part 2, p. 271, Hippokrates Verlag, Stuttgart 1980.
[3] D. Barron, M. Kaouadji, and A. M. Mariotte, Z. Naturforsch. **39c,** 167 (1984).
[4] T. Yamagishi and H. Kaneshima, Yakugaku Zasshi **97,** 237 (1977).
[5] L. F. Bjeldanes and I. S. Kim, J. Org. Chem. **42,** 2333 (1977).
[6] M. J. M. Gijbels, J. J. C. Scheffer, and A. Baerheim Svendsen, Planta Med. **44,** 207 (1982).
[7] S. K. Banerjee, B. D. Gupta, W. S. Sheldrick, and G. Höfle, Liebigs Ann. Chem. **1982,** 699.
[8] M. Kaouadji, M. Puech-Baronnat, and A. M. Mariotte, Tetrahedron Lett. **24,** 4675 (1983).
[9] M. Kaouadji, H. Reutenauer, A. J. Chulia, and A. Marsura, Tetrahedron Lett. **24,** 4677 (1983).
[10] M. Cichy, V. Wray, and G. Höfle, Liebigs Ann. Chem. **1984,** 397.