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(+)-NEO-OLIVIL FROM ROOTS OF *URTICA DIOICA*

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Key Word Index—*Urtica dioica*; Urticaceae; roots; lignan; (+)-neo-olivil; (−)-pinoresinol; (−)-epipinoresinol.

Abstract—The all-*trans* configuration of (+)-neo-olivil from *Urtica dioica* was established by NMR-spectroscopy. After transforming it to (−)-epipinoresinol and (−)-pinoresinol with trimethylorthoformate and H_2SO_4 the absolute configuration was determined as 7*R*,7′*R*,8*S*,8′*S*. © 1997 Elsevier Science Ltd

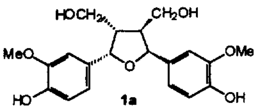
INTRODUCTION

Extracts from roots of *Urtica dioica* L. are used in the treatment of benign prostatic hyperplasia [1]. There is some evidence that the presence of lignans is responsible for the positive effect of this drug [2]. The main lignan in the roots of *U. dioica* is neo-olivil (**1**) [3]. The structural formula of neo-olivil was first presented by B. Vanzetti *et al.* in 1937 [4]. It was later revised by K. Freudenberg [5]. In 1981, A. Hernandez isolated (−)-neo-olivil from *Thymus longiflorus* as its tetra-acetate. Based on the NMR data, he deduced a *cis-trans-cis* configuration (**1a**) [6]. Later publications dealing with neo-olivil all refer to this structure [3, 7]. Our experiments show that this structure needs revision.

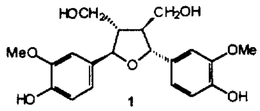
RESULTS AND DISCUSSION

A water–methanol extract of roots of *U. dioicae* which is the basis of pharmaceutical applications, was investigated for lignans. As most of the lignans occur in glycosidic forms, glycosidic bonds were cleaved with cellulase.

The aqueous hydrolysate was defatted with cyclohexane and then extracted with ethyl acetate. The crude ethyl acetate extract was separated by preparative- and semi-preparative HPLC on RP-18. After a two step chromatography, 8.3 mg of pure neo-olivil were obtained as a white powder with an optical rotation of $[\alpha]_D^{23} = +49°$. The structure of neo-olivil and its tetra-acetate was elucidated by EI-MS and NMR-spectroscopy. The mass spectral, ^{13}C and ^1H NMR data are in agreement with the data given by Hernandez and Chaurasia. The optical rotation and the symmetry required by ^1H and ^{13}C NMR make an all-*trans* **1** and a *cis-trans-cis* **1a** structure possible [6]. To decide between these structures, a ROESY experiment was performed [8]. The ROESY cross-peaks observed between H-2/6 and H-8 and between H-9 and H-7 are only in agreement with the all-*trans* structure **1** (Fig. 1). This revises all earlier publications.



1a



1

The absolute configuration was established by conversion of **1** to compounds of known absolute configuration. 12 mg crude (+)-neo-olivil on treatment with 15 ml trimethylorthoformate–H_2SO_4 yielded compound **2** and **3**, which were separated by HPLC. The high resolution mass spectra of both **2** and **3** fits to $C_{20}H_{22}O_6$, which indicates a loss of water from **1**. By use of EI-mass spectrometry, ^1H and ^{13}C NMR, **2** and **3** were identified as pinoresinol [9, 10] and epipinoresinol [11]. This new reaction, which can be used

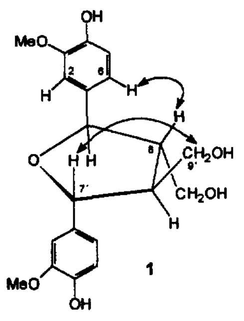


Fig. 1. Selected ROESY-cross-peaks of (+)-neo-olivil (**1**)

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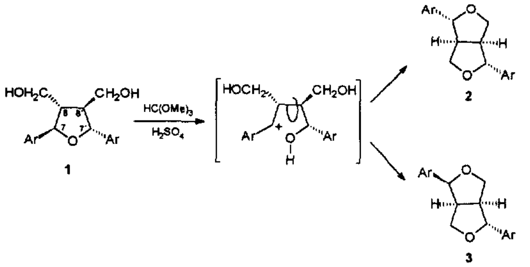

Fig. 2. Formation of (−)-pinoresinol (**2**) and (−)-epipinoresinol (**3**)

to transform 1,4-diols to oxolanes, is the chemical proof of the *trans*-configuration of C-9 and C-9′. To form the bisepoxylignans **2** and **3** the ether linkage of (+)-neo-olivil was cleaved and after rotation of the bond between C-8-C-8′ it has been closed again (Fig. 2). After trimethylsilylation, the reaction products were analysed by GC mass spectrometry. Beside pinoresinol and epipinoresinol $[M]^+ = m/z$ 502 traces of compounds $[M]^+ = m/z$ 562 and $[M]^+ = m/z$ 622 were observed, giving evidence for the removal of water via a transesterification of trimethylorthoformate. However, the isomerisation on C-7 makes an ionic intermediate probable.

The optical rotatory power of both compounds is (−) and in agreement with the literature [12, 13], which indicates an *S* configuration at C-8 and C-8′. Regarding the relative configuration of neo-olivil, the absolute stereochemistry of (+)-neo-olivil must be 7,7′*R*, 8,8′*S*.

EXPERIMENTAL

Extractum Radius Urticae (Finzelberg, Andernach, Germany) H_2O–MeOH (4:1) CH. B. 1313200. NMR: Bruker DRX 500; EIMS, HRMS: MAT 8500 (Finnigan); GC (H_2 at 50 kpa, 3 min 80°, 80° to 280° with 3° min^{-1}, 280° for 15 min) Carlo Erba GC using a fused-silica capillary column coated with DB 1 phase (30 m × 0.32 mm, film 0.1 μm, J and W Scientific). R_i was calcd according to van den Dool *et al.* [14]. Prep. HPLC: Li Chroprep RP 18, 5–10 μm (240 mm × 20 mm); semiprep. HPLC: Spherisorb ODS-2, 5 μm (240 mm × 8 mm), detection in both cases at 280 nm.

Trimethylsilylation with *N*-methyl-*N*-trimethylsilyltrifluoracetamide (Macherey and Nagal, Düren, Germany) 8 hr at room temp. Optical rotatory power: Perkin–Elmer 241 I = 10 cm.

(+)-*Neo-olivil.* 200 g Extractum Radix Urticae were dispersed in 500 ml H_2O and 1 g Cellulase CT (Merck) added. After stirring for 2 days the aq. soln was extracted with cyclohexane and EtOAc (300 ml each × 2). The EtOAc extract was directly used for prep. HPLC (MeOH–H_2O; flow 18 ml min^{-1}, gradient 20% MeOH to 50% MeOH in 30 min, R_t 10.4 min). Semiprep. HPLC of the crude neo-olivil (MeCN–H_2O; flow 2 ml min^{-1}, isocratic 20% MeCN; $R_t = 13$ min) gave 8.3 mg pure (+)-neo-olivil. GC (trimethylsilylated): $R_i = 3230$; EIMS 70 eV, m/z (rel. int.): 376 $[M]^+$ (66), 224 (34), 196 (61), 194 (12), 193 (88), 180 (42); 176 (63), 175 (100), 151(29), 137 (44); HRMS fits 100% to $C_{20}H_{24}O_7$ (376.152205); ^{1}H NMR (500 MHz, CD_3OD): δ 2.31 (2H, *m*, H-8/8′), 3.60 (2H, *dd*, $J = 11.3$ Hz, $J = 5.5$ Hz, H_a-9/9′), 3.69 (2H, *dd*, $J = 11.3$ Hz, $J = 3.7$ Hz, H_b-9/9′), 3.87 (6H, *s*, OMe), 4.92 (2H, *d*, *br*, $J = 8$ Hz, H-7/7′), 6.78 (2H, *d*, $J = 8$ Hz, H-5/5′), 6.87 (2H, *dd*, $J = 8$ Hz, $J = 2$ Hz, H-6/6′), 7.02 (2H, *d*, $J = 2$ Hz, H-2/2′); ^{13}C NMR (125.75 MHz): δ 55.4 (C-8/8′), 56.4 (OMe), 61.7 (C-9/9′), 84.4 (C-7/7′), 111.1 (C-2/2′), 116.0 (C-5/5′), 120.5 (C-6/6′), 134.9 (C-1/1′), 147.6 (C-4/4′), 149.1 (C-3/3′). ROESY (phase sensitive using TPPI, spinlock cw pulse 250 ms) cross-peaks between H 2/6 and H-8 and between H-9 and H-7. $[\alpha]_D^{23} = +49°$ (EtOH, *c* 0.159).

(+)-*Neo-oliviltetraacetate.* 3 mg (+)-neo-olivil were dissolved in 2 ml pyridine and 0.5 ml Ac_2O. After 12 hr the solvent was removed *in vacuo*. GC: $R_i = 3490$; EIMS 70 eV, m/z (rel. int.): 544 $[M]^+$ (6), 502 (3), 484 (9), 442 (14), 425 (10), 238 (14), 222 (100), 179 (13), 178 (78), 175 (16), 151 (24), 150 (12). HRMS fits 100% to $C_{28}H_{32}O_{11}$ (544.194465); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.97 (6H, *s*, $CH_2OCOC\underline{H}_3$), 2.29 (6H, *s*, $ArOCOC\underline{H}$), 3.80 (6H, *s*, OMe), 4.23 (4H, *d*, $J = 5.1$ Hz, H-9/9′), 4.96 (2H, *d*, $J = 7.5$ Hz, H-7/7′), 6.94 (2H, *dd*, $J = 8$ Hz, $J = 2$ Hz, H-6/6′), 7.01 (2H, *d*, $J = 8$ Hz, H-5/5′), 7.03 (2H, *d*, $J = 2$ Hz, H-2/2′); ^{13}C NMR (75.45 MHz, $CDCl_3$): δ 20.6 ($CH_2OCO\underline{C}H_3$), 20.7 ($ArOCO\underline{C}H_3$), 50.4 (C-8/8′), 56.0 (OMe), 63.4 (C-9/9′), 82.6 (C-7/7′), 110.0 (C-2/2′), 118.0 (C-6/6′), 127.7 (C-5/5′), 139.4 (C-1/1′), 140.0 (C-4/4′), 151.2 (C-3/3′), 168.6 ($CH_2O\underline{C}OCH_3$), 170.4 ($ArO\underline{C}OCH_3$). $[\alpha]_D^{23} = +17°$ (EtOH, *c* 0.065).

Dehydration. 12 mg crude (+)-neo-olivil were dissolved in 5 ml trimethylorthoformate (Merck). 20 μl H_2SO_4 (96%) were added, the mixt. heated to 100° for 2 hr and stirred overnight. After neutralisation with satd $NaHCO_3$ the soln was diluted with 50 ml H_2O and extracted (× 2) with 100 ml CH_2Cl_2. The two main compounds were sepd by semiprep. HPLC (Flow 2 ml min^{-1}, solvent MeCN–H_2O, gradient 20% MeCN to 50% MeCN in 30 min).

(−)-*Pinoresinol.* HPLC: $R_t = 14$ min; GC (trimethylsilylated): $R_i = 3257$; HRMS fits 100% to $C_{20}H_{22}O_6$ (544.194465). EIMS 70 eV, m/z (rel. int): 358 $[M]^+$ (84), 327 (13), 180 (16), 163 (39), 153 (12), 152 (42), 151 (100), 150 (33), 137 (64), 131 (34); ^{1}H and ^{13}C NMR fits to ref. [8]. $[\alpha]_D^{23}$ = fits to ref. [11].

(−)-*Epipinoresinol.* HPLC: $R_t = 15.4$ min; GC: $R_i = 3247$; EIMS 70 eV, m/z (rel. int.): 358 $[M]^+$ (88), 180 (16), 163 (31), 153 (10), 152 (42), 151 (100), 150 (23), 137 (56), 131 (23); ^{1}H and ^{13}C NMR fits to ref. [10]. $[\alpha]_D^{23}$ = fits to ref. [12].

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