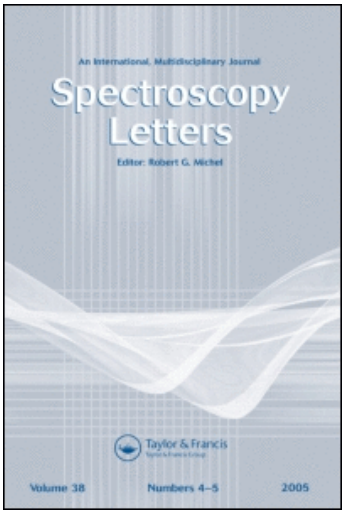

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NMR Spectral Data of Benzofurans and Bithiophenes from Hairy Root Cultures of Tagetes Patula Andthe Molecular Structure of Isoeuparin

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NMR SPECTRAL DATA OF BENZOFURANS AND BITHIOPHENES FROM HAIRY ROOT CULTURES OF *TAGETES PATULA* AND THE MOLECULAR STRUCTURE OF ISOEUPARIN

Key Word Index -- *Tagetes patula*; Asteraceae; hairy root cultures; benzofurans; bithiophenes; ^{1}H NMR.

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Abstract -- In the course of our biosynthetic studies of constituents of hairy root cultures of *Tagetes patula* , we isolated four bithiophenes, [5-(3-buten-1-ynyl)]-2,2'-bithiophene (BBT), 5-(4-acetoxy-1-butynyl)-2,2'-bithiophene (BBTOAc), 5-(4-hydroxy-1-butynyl)-2,2'-bithiophene (BBTOH) and 5-(3,4-diacetoxy-1-butynyl)-2,2'-bithiophene [BBT(OAc)$_2$]. In addition, stigmasterol, β-farnesene, three known benzofurans, of which only one was previously isolated from *T. patula*, as well as a new benzofuran were obtained. The structures of the compounds were determined by spectral analysis and comparison with published data. The molecular structure of the benzofuran isoeuparin was established by single crystal x-ray diffraction.

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†Deceased

INTRODUCTION

Hairy root cultures are known to produce secondary metabolites characteristic of the species from which they are derived [1]. Previous investigations of hairy root cultures and callus tissue cultures of *Tagetes patula* (Asteraceae) [2-5] gave the known bithiophenes, BBT (**7**), BBTOAc (**8**), BBTOH (**6**), $BBT(OAc)_2$ (**5**) and the known benzofurans, isoeuparin (**1**) and euparin. Because of the wide range of biological activities of thiophenes [6] and minor variations in the chromatographic patterns of different batches of hairy root cultures of *T. patula*, we continued our chemical investigation of various hairy root cultures which were obtained during our biosynthetic studies of hairy root constituents of *T. patula* [7,8].

We report below high field ^1H NMR data of compounds **2-6** as well as the molecular structure of isoeuparin (**1**), which was determined by single crystal X-ray diffraction.

RESULTS AND DISCUSSION

Chemical data.

Vacuum liquid chromatography (VLC) [9] of the dichloromethane extract of hairy root cultures of *Tagetes patula*, followed by preparative TLC (prep. TLC) gave compounds **1** and **3** to **10**. Isoeuparin (**1**) had been previously isolated from hairy root cultures of *T. patula* [2,3] and synthesized [10]. Its molecular structure, shown in Figure 1, was established by single crystal X-ray analysis and the coordinates are summarized in Table 3.

The ^1H NMR and ^{13}C NMR data of dehydrotremetone (**2**) have been previously reported [11]. In the ^1H NMR spectrum the chemical shifts of the two methyl groups (C-11 and C-14) were reported to absorb at δ 2.22 and 1.80, respectively [11]. In our sample, the ketone methyl (C-11) absorbed at δ 2.66 and the vinyl methyl (C-14) appears at δ 2.14.

Compound **3** was a yellow oil which exhibited in its ^1H NMR spectrum signals typical of a benzofuran with a methyl ketone and a hydrogen bonded hydroxyl group. The position of the H-bonded hydroxyl group must be at C-6 because the two aromatic ^1H NMR signals appeared as singlets at δ 7.50 and 6.37 and therefore belong to protons H-4 and H-7, respectively. Two one-

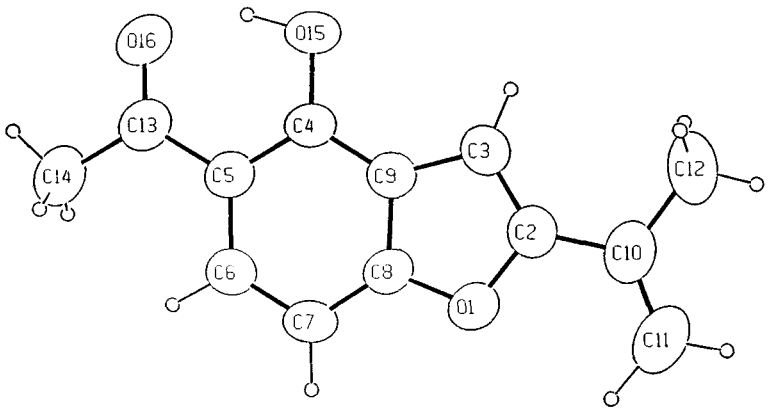
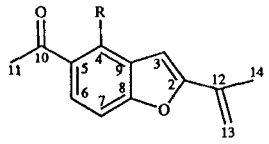


Figure 1. The molecular structure of isoeuparin (**1**).

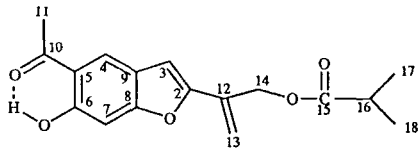
proton doublets at δ 4.71 (J = 13.6 Hz) and δ 4.63 (J=14.0 Hz) as well as a six-proton doublet at δ 0.94 (J=6.4 Hz) and an one-proton multiplet at δ 2.10 suggested the presence of an isobutyrate ester moiety. This was confirmed by the mass spectral data which showed the molecular ion at *m/z* 304 and a strong peak at *m/z* 216 caused by the loss of isobutyric acid. The ^{13}C NMR spectrum also supported structure **3**. Compound **3** had been previously reported as a constituent of *Doronicum paralianclies* [12]. The ^{1}H NMR parameters of this compound were not reported by the authors and the reference to its ^{1}H NMR data [13] were those of a related compound with a hydroxy group at C-4 instead of C-6. Therefore, we report in Table 1 the assignments of the ^{1}H NMR spectral data of compound **3**, and its ^{13}C NMR spectral data are listed in the experimental section.

The new benzofuran, 14-isobutyloxyeuparin (**4**), exhibited ^{1}H NMR signals similar to those of **3** with an additional olefinic signal at δ 7.00 in the ^{1}H NMR spectrum suggesting a C2-C3 double bond in **4**. The signal at δ 12.5 indicated a hydrogen-bonded hydroxyl group at C-6 due to the appearance of two singlets at δ 7.94 (H-4) and δ 6.64 (H-7).

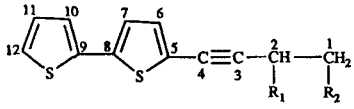
The two bithiophenes BBT(OAc)$_2$ (**5**) and BBTOH (**6**) were previously isolated from the roots of *Echinops sphaerocephalus* [14] and also detected by HPLC in root cultures of *T. patula* [4,5]. We report here high field ^{1}H NMR spectral data of compounds **5** and **6**. The ^{1}H NMR



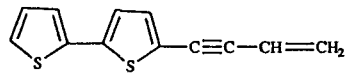
1 R = OH
2 R = H



3 2,3-dihydro
4



5 R_1, R_2 = OAc
6 R_1 = H, R_2 = OH
8 R_1 = H, R_2 = OAc



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spectrum of BBT(OAc)$_2$ (**5**) exhibited two methyl singlets at δ 2.11 and δ 2.15 as well as three one-proton doublets of a doublet at δ 4.43 (J=11.6, 7.3 Hz), δ 4.31 (J=11.6, 3.7 Hz) and δ 5.86 (J=7.3, 3.7 Hz). The chemical shift and the coupling constants of the above three one-proton signals suggested the presence of two adjacent acetate moieties. This was supported by a molecular ion m/z = 334 in the mass spectrum of **5**. The five aromatic signals in **5** (Table 2) were similar to the aromatic thiophene proton signals of BBTOAc (**8**) [3]. The ^{1}H NMR spectrum of BBTOH (**6**) showed aromatic thiophene proton signals similar to those of **5**. The presence of two vicinal methylene triplets at δ 3.82 (2H-1) and δ 2.73 (2H-2), and the chemical shift at δ 3.82 were in agreement with a hydroxyl group at C-1. The molecular ion (m/z = 234) and a peak at m/z = 203 (M^+-CH_2-OH) in the mass spectrum confirmed the presence of a hydroxyl group at C-1 in BBTOH (**6**).

EXPERIMENTAL

Hairy root cultures.

Hairy root cultures of *Tagetes patula* were obtained as previously described [7].

Table 1. ^1H NMR spectral data of compounds **2***, **3** and **4** (200 MHz, $CDCl_3$, $CDCl_3$, as int. standard).

H	2	3	4
2	-----	5.36bt	-----
3	6.67s	3.37dd	7.00s
3'	-----	3.09dd	-----
4	8.18d	7.50s	7.94s
6	7.94dd	-----	-----
7	7.48d	6.37s	6.64s
11	2.66s	2.54s	2.69s
13	5.24d	5.33s	5.99s
13'	5.83s	5.27s	5.51s
14	2.14bs	4.71d	4.94bs
14'	-----	4.63d	4.94bs
16	-----	2.10m	2.07m
17	-----	0.94d	0.96d
18	-----	0.94d	0.96d
OH	-----	12.96s	12.50bs

*Data obtained at 400 MHz

Coupling constants in Hz or line separations: compound **2**: 4=1.7, 6=8.8, 1.8, 7=8.6, 13=1.3; compound **3**: 2=9.7, 7.6, 3=15.2, 9.7, 3'=15.4, 7.6; 14=14'=13.6, 14'=14.0, 17,18=6.4; compound **4**: 17,18=6.1.

Extraction and isolation.

Hairy roots of *Tagetes patula* (48.4 g of dry wt) were soaked in 800 ml of CH_2Cl_2 for 24 hrs. After suction filtration and evaporation of the solvent in vacuo, the crude extract (1.17 g) was subjected to VLC [9] using hexane, CH_2Cl_2 and ethyl acetate (EtOAc) mixtures of increasing polarity, yielding twelve 50 ml fractions. Fraction 5 (26 mg) was re-chromatographed by prep. TLC using hexane: EtOAc (10:1) yielding 15 mg of compound **1**. Prep. TLC separation of

Table 2. ^{1}H NMR spectral data of BBT(OAc)$_2$ (**5**) (400 MHz, $CDCl_3$) and BBTOH (**6**) (200 MHz, $CDCl_3$) with $CDCl_3$ as internal standard.

H	5	6
1	4.43dd	3.82t
1'	4.31dd	3.82t
2	5.86dd	2.73t
6	7.15d	7.05d
7	7.02m	7.00d
10	7.18d	7.16d
11	7.02m	7.02d
12	7.25dd	7.23bd
OAc	2.11s, 2.15s	-----

Coupling constants in Hz or line separations: compound **5**: 1=11.6, 7.3, 1'=11.6, 3.7, 2=7.3, 3.7, 6=4.0, 10=3.6, 12=5.1, 1.2; compound **6**: 1=6.1, 2=6.3, 6=3.9, 7=3.9, 10=3.5, 11=4.4, 12=5.1.

fraction 8 afforded compounds **3**-**6**. After prep. TLC separation, fraction 9 gave 13 mg of stigmasterol (**10**).

Compound **2** was obtained from hairy roots of *T. patula* fed with ^{13}C-labeled acetate precursors [7]. VLC of the CH_2Cl_2 extract followed by prep. TLC gave 1 mg of compound **2**.

X-ray data of isoeuparin (**1**)

A crystal of dimensions 0.08 x 0.23 x 0.55 mm was used for data collection on an Enraf-Nonius CAD4 diffractometer equipped with CuKα radiation (λ = 1.54184 Å) and a graphite monochromator. Crystal data are: $C_{13}H_{12}O_3$, Mr = 216.2, monoclinic space group C2/c, a = 13.6359 (14), b = 12.209 (2), c = 14.924 (2) Å, β = 118.635(9)°, V = 2180.7 (5) Å^3, Z = 8, d_c = 1.317 g/cm^3, T = 22°C. Intensity data were measured by ω-2θ scans of variable rate designed to

Table 3. Positional parameters of isoeuparin (**1**) and their estimated standard deviations.

Atom	x	y	z	$B_{eq}(Å^2)$
C1	0.37698(6)	0.11330(6)	0.03263(6)	4.49(2)
C2	0.33674(8)	0.00696(9)	0.00924(7)	4.21(2)
C3	0.41519(8)	-0.06523(9)	0.06908(8)	4.18(2)
C4	0.61855(8)	-0.02896(9)	0.21482(7)	3.80(2)
C5	0.69332(7)	0.05716(8)	0.26480(7)	3.92(2)
C6	0.65930(8)	0.16633(9)	0.23474(8)	4.33(2)
C7	0.55466(9)	0.19297(9)	0.15685(9)	4.59(3)
C8	0.48396(7)	0.10539(9)	0.11064(7)	3.97(2)
C9	0.51176(7)	-0.00343(8)	0.13598(7)	3.74(2)
C10	0.22208(9)	-0.0064(1)	-0.07203(8)	5.04(3)
C11	0.1599(1)	0.0800(1)	-0.1200(1)	6.97(4)
C12	0.1804(1)	-0.1218(1)	-0.0944(1)	6.43(4)
C13	0.80664(8)	0.0327(1)	0.34620(8)	4.49(2)
C14	0.8877(1)	0.1227(1)	0.3999(1)	6.16(4)
C15	0.64556(7)	-0.13431(6)	0.23969(6)	5.22(2)
C16	0.83633(7)	-0.06338(7)	0.37100(7)	5.77(2)

yield $I = 25\sigma(I)$ for all significant reflections. One hemisphere of data was collected within the limits $2° < \theta < 75°$. Data reduction included corrections for background, Lorentz, polarization, decay (4.6%) and absorption. Absorption corrections ($\mu = 7.3$ cm^{-1}) were based on psi scans, with minimum relative transmission coefficient 89.40%. A total of 4798 data were measured; redundant data were averaged to yield 2183 unique data; 1797 had $I > 3\sigma(I)$ and were used in the refinement.

The structure was solved by direct methods and refined by full-matrix least squares, treating nonhydrogen atoms anisotropically, using Enraf-Nonius SDP [15]. Hydrogen atoms were located in difference maps and refined isotropically. Convergence was achieved with R = 0.037 and Rw = 0.053. Coordinates are given in Table 3, and the molecular structure is illustrated in Figure 1.

Isoeuparin (**1**). $C_{13}H_{12}O_3$; EIMS *m/z* (rel. int.): 216 $[M]^+$ (95), 201 $[M-Me]^+$ (100), 198 $[M-H_2O]^+$ (16), 173 (13), 145 (4), 117 (21), 91 (30), 77 (21), 63 (25), 51 (37), 39 (73); X-ray parameters are summarized in Table 3.

Dehydrotremetone (**2**). $C_{13}H_{12}O_2$; yellow oil, EIMS *m/z* (rel. int.): 200 $[M]^+$ (2), 185 $[M-Me]^+$ (0.7), 88 (17), 86 (100), 84 (74), 49 (42), 50 (51), 35 (25); ^1H NMR data in Table 1.

2,3-*dihydro*-14-*isobutyryloxyeuparin* (**3**). $C_{17}H_{20}O_5$; yellow oil; EIMS *m/z* (rel. int.): 304 $[M]^+$ (0.2), 216 [M-isobutyric acid]$^+$ (45), 201 [216-Me]$^+$ (6), 176 (100), 161 [176-Me]$^+$ (34); 57 (27); ^1H NMR in Table 1; ^{13}C NMR (50 MHz), $CDCl_3$): 22.83, 98.34, 113.97, 114.88, 118.15, 126.73, 142.40, 165.79, 166.16, 172.57, 202.00.

14-*isobutyryloxyeuparin* (**4**). $C_{17}H_{18}O_5$; yellow oil; EIMS *m/z* (rel. int.): 302 $[M]^+$ (16), 301 (100) 214 [M-isobutyric acid]$^+$ (5), 199 [214-Me]$^+$ (5), 174 (3), 57 (22); ^1H NMR in Table 1.

5-(3,4-*diacetoxy*-1-*butynyl*)-2,2'-*bithiophene* (**5**). $C_{16}H_{14}O_4S_2$; yellow oil; EIMS *m/z* (rel. int.): 334 $[M]^+$ (25); 274 [M-AcOH]$^+$ (1), 232 (45), 95 (4), 73 (5), 43 (100); ^1H NMR in Table 2.

5-(4-*hydroxy*-1-*butynyl*)-2,2'-*bithiophene* (**6**). $C_{12}H_{10}OS_2$; yellow oil; *m/z* (rel. int.): 234 $[M]^+$ (48), 203 $[M-CH_2OH]^+$ (4), 134 (17), 95 (100), 82 (30), 75 (17), 62 (26), 34 (22); ^1H NMR in Table 1.

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