# Antibiotic Activity and Absolute Configuration of 8S-Heptadeca-2(Z),9(Z)-diene-4,6-diyne-1,8-diol from Bupleurum salicifolium

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# ANTIBIOTIC ACTIVITY AND ABSOLUTE CONFIGURATION OF 8*S*-HEPTADECA-2(*Z*),9(*Z*)-DIENE-4,6-DIYNE-1,8-DIOL FROM *BUPLEURUM SALICIFOLIUM*

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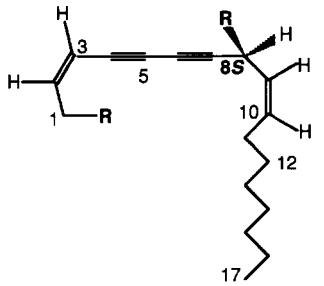
ABSTRACT.—A polyacetylene has been isolated from *Bupleurum salicifolium*. Its structure and absolute configuration were determined to be 8*S*-heptadeca-2(*Z*),9(*Z*)-diene-4,6-diyne-1,8-diol [**1**] by means of $^1$H- and $^{13}$C-nmr spectroscopic studies, including $^1$H-$^{13}$C heteronuclear correlation (HMQC) and long-range correlation spectra with inverse detection (HMBC). Its absolute configuration was determined by application of the Horeau method. This compound exhibited significant antibiotic activity against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. Also isolated during this investigation were the known compounds: betulin, herniarin, 6,7,8-trimethoxycoumarin, *p*-hydroxyphenethyl alcohol, pluviatolide, guamaroline, bursehernin, guayadequiol, kaerophyllin, and matairesinol dimethyl ether.

As part of a search for new bioactive compounds from *Bupleurum salicifolium* Soland (Umbelliferae) (1–6), a species endemic to the Canary Islands, we isolated an unsaturated product [**1**]. Unsaturated compounds isolated from the *Bupleurum* genus tend to be optically inactive with 14 and 15 carbon atoms (7) although some optically active $C_{15}$ compounds, with as yet unknown absolute configuration, have also been obtained (8). Bohlmann *et al.* (9) isolated a polyacetylene from *Azorella trifurcata* (Umbelliferae) which yielded similar $^1$H-nmr data to those of **1**. Although the authors did not publish data concerning the coupling constants, optical activity, $^{13}$C nmr or absolute configuration, biogenetic considerations make it probable that both compounds have the same absolute configuration. However, they might also be two enantiomeric isomers.

In this paper we report on the isolation, unequivocal assignments of $^1$H- and $^{13}$C-nmr spectra, and absolute configuration of the first $C_{17}$ polyacetylene isolated from the genus *Bupleurum*. This compound proved to have antibiotic activity against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*.




**1** R=OH
**2** R=OAc

A cold EtOH extract of the leaves of *B. salicifolium* was repeatedly chromatographed on Si gel and Sephadex LH-20 to give the following products: the polyacetylenic compound [**1**], the triterpene betulin, the two coumarins herniarin (10) and 6,7,8-trimethoxycoumarin (11), *p*-hydroxyphenethyl alcohol and the known lignans pluviatolide, guamaroline, bursehernin, guayadequiol, kaerophyllin, and matairesinol dimethyl ether (3–5).

The polyacetylene **1** was isolated as an optically active oil ($[\alpha]^{20}D$ +270°, $c$=0.7, $CHCl_3$) and its uv spectrum revealed a typical ene-diyne (12) chromophore absorption (λ max (EtOH) 316, 288, 270, 228 nm). The most significant signals in its $^1$H-nmr spectrum ($CDCl_3$) (Table 1) were a triplet at δ 0.88 ($J$=6.7 Hz, 3H) assigned to a methyl group

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TABLE 1. $^1$H-Nmr (200 MHz) Data of **1** and **2**.[a]

| Proton | Compound | | | |
|---|---|---|---|---|
| | $CDCl_3$ | | $C_6D_6$ | |
| | **1** | **2** | **1** | **2** |
| 1 ..... | 4.42 dd (6.4, 1.2) | 4.82 dd (6.5, 1.5) | 4.14 dd (6.2, 1.5) | 4.62 dd (6.5, 1.5) |
| 2 ..... | 6.26 dt (10.9, 6.4) | 6.10–6.30 m | 5.87 dt (11.1, 6.3) | 5.70 dt (11.1, 6.5) |
| 3 ..... | 5.40–5.60 m | 5.66–5.72 m | 5.22 d (11.1) | 5.17 d (11.4) |
| 8 ..... | 5.25 d (7.4) | 6.10–6.30 m | 5.16 d (8.2) | 6.49 d (8.5) |
| 9 ..... | 5.40–5.60 m | 5.49 dd (10.4, 10.4) | 5.56 dd (10.6, 8.2) | 5.56 dd (10.5, 10.5) |
| 10 ..... | 5.40–5.60 m | 5.66–5.72 m | 5.36 dt (10.7, 7.4) | 5.41 dt (10.7, 7.0) |
| 11 ..... | 2.12 q (6.8) | 2.16 q (6.7) | 1.88 q (6.7) | 2.00 q (6.9) |
| 12–16 ..... | 1.10–1.50 br s | 1.10–1.50 br s | 1.15–1.50 br s | 1.10–1.40 br s |
| 17 ..... | 0.88 t (6.7) | 0.88 t (6.9) | 0.89 t (6.5) | 0.89 t (6.4) |
| OH ..... | 2.31 s | | 2.11 s | |
| OH ..... | 1.94 s | | 2.11 s | |
| OAc ..... | | 2.08 s | | 1.57 s |
| OAc ..... | | 2.08 s | | 1.58 s |

[a]Values in δ; coupling constants (Hz) in parentheses.

linked to a -$CH_2$-group, a broad singlet between δ 1.10 and 1.50 (10H) for 5× -$CH_2$-, a quartet at δ 2.12 ($J$=6.8 Hz, 2H), a doublet of doublets at δ 4.42 ($J$=1.2 and 6.4 Hz, 2H) attributed to a methylene group linked to an oxygen, a multiplet between δ 5.40 and 5.60 corresponding to three olefinic hydrogens, and a signal at δ 6.26 (dt, $J$=10.9 and 6.4 Hz, 1H) characteristic of an olefinic hydrogen linked to a $CH_2$ and cis to the other double-bond hydrogen.

The hrms spectrum of **1** gave a molecular formula of $C_{17}H_{24}O_2$ and the $^{13}$C-nmr spectrum (Table 2) and a DEPT nmr experiment revealed four quaternary carbons between δ 68.7 and 83.4, typical of acetylenic carbons. To obtain more information about the relative positions of these groups in the molecule of **1**, a COSY experiment was carried out and the following observations were made: coupling of the signal at δ 6.26 with the dd at δ 4.42, establishing that unit $OHCH_2$-CH=CH- is one of the terminal fragments of the molecule; coupling of the doublet at δ 5.25 with the multiplet of signals associated with the three olefinic hydrogens, which established that the -CHOH was coupled to one of the two double bonds of the molecule (i.e., -CHOH-CH=CH-), coupling of the triplet at δ 0.88 with the broad singlet attributed to 5× -$CH_2$-, and coupling of the quartet at δ 2.12 with the multiplet at δ 5.40–5.60 corresponding to the olefinic hydrogens and the broad singlet, thus defining the other terminal fragment of the molecule as $CH_3$-$(CH_2)_6$-CH=CH-. HMQC nmr results were used in assigning one-bond proton-carbon relationships and they were especially helpful to assign the C-2 and C-11 signals. HMBC analysis (Table 3) determined the assignments of the methylene carbons C-12, C-13, C-15, and C-16, and showed that the doublet of triplets at δ 6.26 due to H-2 was clearly three-bond coupled with one of

TABLE 2. $^{13}$C-Nmr (50 MHz) Data ($CDCl_3$) of **1** and **2**.[a]

| Carbon | Compound | | Carbon | Compound | |
|---|---|---|---|---|---|
| | **1** | **2** | | **1** | **2** |
| 1 | 60.37 | 62.69 | 11 | 27.39 | 28.27 |
| 2 | 145.84 | 141.18 | 12 | 28.83 | 29.50 |
| 3 | 108.49 | 111.51 | 13 | 28.93 | 29.50 |
| 4 | 74.39 | 74.64 | 14 | 29.06 | 29.50 |
| 5 | 78.67 | 79.82 | 15 | 31.55 | 32.17 |
| 6 | 83.40 | 80.82 | 16 | 22.34 | 23.00 |
| 7 | 68.67 | 69.97 | 17 | 13.84 | 14.46 |
| 8 | 58.07 | 60.62 | OAc | | 169.89 |
| 9 | 127.62 | 124.14 | | | 21.15 |
| 10 | 133.66 | 136.79 | OAc | | 171.03 |
| | | | | | 21.31 |

[a]Values based on HMQC, HMBC, and DEPT experiments. Chemical shifts are given in δ.

the acetylene carbons, and enabled the structure of **1** to be proposed.

Acetylation of **1** with $Ac_2O$/pyridine formed the corresponding acetyl derivative **2**, which confirmed the above data (see Tables 1 and 2). The stereochemistry of the double bond between carbons 9 and 10 was established as *Z* from the value of the coupling constant ($H_9$-$H_{10}$) (13) in the $^1$H-nmr spectrum (see Table 1). The absolute configuration of **1** was determined as 8*S* by application of the Horeau method (14) which has been used for analogous compounds (15,16). The asymmetric esterification of **1** with a racemic mixture of α-phenylbutyric anhydride afforded (+)-α-phenylbutyric acid in an optical yield of 9%.

The polyacetylene **1** showed antibiotic activity against two Gram-positive bacteria (*S. aureus* and *B. subtilis*), but was inactive when tested against Gram-negative bacteria (*Escherichia coli, Salmonella* sp., *Pseudomonas aeuriginosa*) and the yeast *Candida albicans*. The MIC (minimum inhibitory concentration) was determined following the method of Buttiaux *et al.* (17) and was established as 10 μg/ml against *S. aureus* and 10.5 μg/ml against *B. subtilis*, respectively.

TABLE 3. Three-Bond $^1$H-$^{13}$C Couplings (HMBC) for Compound **1**.

| Irradiated Proton | Observed Carbons |
|---|---|
| H-1 | C-2[a], C-3 |
| H-2 | C-1[a], C-4, C-3[a] |
| H-8 | C-7[a], C-6, C-10 |
| H-11 | C-12[a], C-13, C-9, C-10[a] |
| H-17 | C-15, C-16[a] |

[a]Two-bond coupling enhancement observed.

The phylogenetic significance of the presence of a $C_{17}$ polyacetylene within the genus *Bupleurum* is not known, although we suppose it could be derived biogenetically from crepenynic acid, which is a precursor of some $C_{17}$ polyacetylenes (12).

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were taken on a Perkin-Elmer 681 spectrophotometer. $^1$H- and $^{13}$C-nmr spectra were measured on a Bruker W-200SY spectrometer using TMS as internal reference. The HMBC and HMQC nmr spectra were run on a Bruker AMX (400 MHz) instrument. Optical rotations were measured on a Perkin-Elmer 550-SE polarimeter. Eims were recorded on VG Micromass LTD-ZAB-2F and Hewlett-Packard 5930-A mass spectrometers at 70 eV. Uv spectra were collected on a Perkin-Elmer model 550-SE instrument. Schleicher-Schüll F-100/LS 254 and prep. tlc 1510/LS 254 plates were used for tlc and Si gel (0.2–0.63 mm) and Sephadex LH-20 were used for cc.

PLANT MATERIAL.—The plant was collected in the Barranco Rio Badajoz, Güimar, Tenerife, Canary Islands, in August 1988. A voucher specimen is lodged in the TFC file in the Department of Biología Vegetal (Botany) of the Universidad de la Laguna.

EXTRACTION AND ISOLATION.—Dried leaves (3.2 kg) of mature specimens of *B. salicifolium* were extracted with cold EtOH. The EtOH extract was treated with $H_2O$, then with *n*-hexane and afterwards with $C_6H_6$ to afford a dark residue (insoluble in $H_2O$ and *n*-hexane and soluble in $C_6H_6$). This residue (112.8 g) was chromatographed on Si gel using as eluent mixtures of *n*-hexane/EtOAc of increasing polarity. Five fractions, A–E, were separated, and A and B were studied. Fractions A and B were chromatographed on Sephadex LH-20 eluted with *n*-hexane-$CHCl_3$-$CH_3OH$ (2:2:1). Betulin (400 mg), **1** (150 mg), 6,7,8-trimethoxycoumarin (6 mg) and bursehernin (89 mg) were separated from fraction A. Fraction B yielded kaerophyllin (15.9 mg), matairesinol dimethyl ether (200 mg), guayadequiol (9.3 mg), herniarin (5 mg), pluviatolide (2.6 mg), guamaroline (5 mg), and *p*-hydroxyphenethyl alcohol (6 mg).

*8S-Heptadeca-2(Z),9(Z)-diene-4,6-diyne-1,8-diol* [**1**].—Yellow oil; $[\alpha]^{20}D$ +270° (*c*=0.7, $CHCl_3$); uv (EtOH) λ max (ε) 316 (17000), 288 (20000), 270 (14000), 228 (8000) nm; ir ν max (film) 3468, 2931, 2861, 2373, 1634, 1454, 1326, 1268, 1024 $cm^{-1}$; eims *m/z* 260 $[M]^+$ (2), 175 (11), 161 (20), 157 (52), 133 (31), and 129 (100); hreims *m/z* 260.1778 (required for $C_{17}H_{24}O_2$, 260.1770); $^1H$ nmr, see Table 1; $^{13}C$ nmr, see Table 2.

*Acetylation of* **1**.—Compound **1** (22.4 mg) was treated with 0.5 ml of $Ac_2O$ and 2 drops of pyridine at room temperature for 34 h. The acetylated mixture was subjected to prep. tlc with $CHCl_3$ as eluent to afford the acetyl derivative, **2** (18.7 mg); eims *m/z* 344 $[M]^+$ (2), 262 (13), 202 (23), 187 (43), 159 (83), 157 (100), 128 (29), and 115 (28); hreims *m/z* 344.1978 (required for $C_{21}H_{28}O_4$, 344.1980); $^1H$ nmr, see Table 1; $^{13}C$ nmr, see Table 2.

*Determination of Absolute Configuration of* **1** *by the Horeau Method.*—Compound **1** (25.2 mg) (0.097 mmol) was added to a solution of 2-phenylbutanoic anhydride (0.97 μl) (0.2 mmol) in anhydrous pyridine (0.4 ml), and the resulting mixture allowed to stand at room temperature for 16 h. $H_2O$ (1 ml) was then added to effect hydrolysis and the mixture was left to stand for 30 min. The organic acid was titrated with 0.1 N NaOH (4.8 ml) in the presence of $C_6H_6$ (3 ml) and a little powdered phenolphthalein. The mixture was then transferred to a funnel and the pink aqueous basic phase was washed with $CHCl_3$ to remove traces of esters and then acidified with 1N HCl (2 μl). The resulting 2-phenylbutanoic acid was extracted with $C_6H_6$. The $C_6H_6$ extracts were dried over $Na_2SO_4$, filtered, and the solvent removed under vacuum to afford 15.2 mg of (+)-2-phenylbutanoic acid {$[\alpha]^{20}D$ +1.5° (*c*=1.5, $CHCl_3$)}. The optical yield was 9.1%.

ANTIMICROBIAL ASSAYS.—The activity of **1** was tested against Gram-positive (*Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* CECT 39), and Gram-negative bacteria (*Escherichia coli* CECT 99, *Salmonella* sp. CECT 456, *Pseudomonas aeuriginosa* AK 958), and the yeast *Candida albicans* UBC1. The strains were maintained on Nutrient Agar (Oxoid) or Sabouraud Agar (Oxoid). The bacterial cultures were developed in Nutrient Broth (Oxoid) and the yeast culture in YEPD medium, with the composition per liter as follows: yeast extract (Oxoid) 10 g, peptone (Difco) 20 g; glucose 20 g.

The minimal inhibitory concentrations (MIC) were determined in liquid medium according to the method of Buttiaux *et al.* (17) in Nutrient broth (Oxoid) or Sabouraud medium (Oxoid), using overnight cultures as inocula. The initial cellular densities were about $10^5$ v.u./ml. The compound **1** was added in a solution of DMSO and tubes with the same proportions of DMSO were used as controls. Cultures were incubated at 37° on a rotary shaker and growth was measured by viable counting on Nutrient Agar or Sabouraud plates.

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