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CHEMICAL CONSTITUENTS OF THE FLORA OF JORDAN, PART III.[1] MONO-*O*-ACETYL DIPHYLLIN APIOSIDE, A NEW ARYLNAPHTHALENE LIGNAN FROM *HAPLOPHYLLUM BUXBAUMII*

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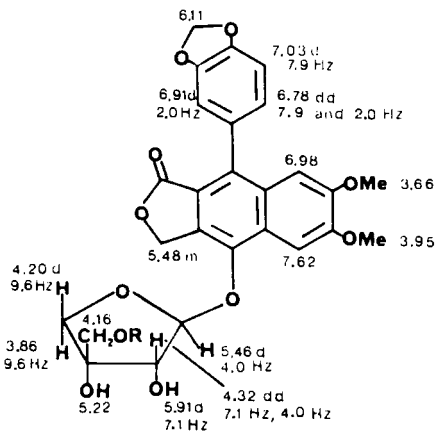
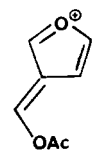
In a continuation of our chemical studies of the constituents of the flora of Jordan, we investigated *Haplophyllum buxbaumii* (Poir.) G. Don fil. (Rutaceae), a yellowish-green perennial herb widespread in northern Jordan. During the course of the present work, *H. buxbaumii* of Turkish origin was reported to contain the quinoline alkaloids kokusaginine, skimmianine, γ-fagarine, 4,5,6-trimethoxyfuroquinoline, 4,5,7-trimethoxyfuroquinoline, and *N*-hydroxymethylflindersine, as well as the lignan justicidin B (2). In the current work, the *H. buxbaumii* collected in Jordan afforded seven compounds, including one new one. These were coumarin (3), the furoquinoline alkaloids dictamnine (4-6) and γ-fagarine (4-6), and four arylnaphthalene lignans. These four were justicidin B (7-9); diphyllin (7,8,10); (−)-tuberculatin [**1**], previously isolated from *Haplophyllum tuberculatum* (11); and the new compound, mono-*O*-acetyl diphyllin apioside [**2**].

Tuberculatin [**1**] and compound **2** both hydrolyze to the lignan diphyllin and the sugar apiose, so compound **2** must be closely related to (−)-tuberculatin [**1**]. The identity of compounds **1** and **2** and the difference between them is shown by ^{1}H nmr.

The 360 MHz (DMSO-d_6) ^{1}H-nmr spectrum of **2** has been summarized around expression **2**. Significantly, a three-proton singlet at δ 2.06 is present, representing an acetyl methyl group, and suggesting that **2** is a mono-*O*-acetyltuberculatin. This was confirmed when acetylation of either (−)-tuberculatin [**1**] or **2** supplied the identical triacetyl derivative that has previously been reported (11). The location of the acetyl function in the apiose moiety of **2** was determined by comparison of the ^{1}H-nmr spectrum of this new lignan with that of **1**. The one-proton triplet at



1 R=H
2 R=Ac (with nmr)



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[1]For Part II, see Abu Safieh *et al.* (1).

δ 5.05 (J=5.5 Hz), due to the primary alcohol hydrogen (CH_2OH) in the spectrum of **1**, is missing in the spectrum of **2**, suggesting acetylation of this hydroxyl. Furthermore, the two-proton multiplet centered at δ 3.45 and representing the methylene protons adjacent to the primary alcohol in the spectrum of (−)-tuberculatin [**1**] is replaced by a two-proton singlet further downfield at δ 4.16 in the spectrum of **2**, in line with acetylation of the primary alcohol function. The assignment of the chemical shifts of the remaining protons in the apiose moiety of **2** was confirmed by double resonance experiments.

Further confirmation of the structure of **2** was obtained from the mass spectrum of the compound which showed a weak molecular ion *m/z* 554 for $C_{28}H_{26}O_{12}$. The base peak, *m/z* 380, corresponds to the diphyllin moiety. A prominent peak is *m/z* 139, which corresponds to ion **a** and which had been previously observed in the mass spectrum of (−)-triacetyltuberculatin (11). This ion is diagnostic of acetylation of the primary alcohol function in the apiose moiety.

The glycosidic linkage in **2** must be of the β-type since this lignan and (−)-tuberculatin [**1**] provided the same levorotatory triacetyl derivative. Additionally, the anomeric proton doublets in the ^{1}H-nmr spectra of **1** and **2** show almost identical chemical shifts, δ 5.45 for **1** and δ 5.46 for **2**, again pointing to similar stereochemistry.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. Ir spectra were recorded on a Perkin-Elmer 577 spectrophotometer. Uv spectra were taken on a PYE Unicam SP-8-500 spectrophotometer. ^{1}H-nmr spectra were recorded on a Bruker 200 MHz or 360 MHz spectrometer either in $CDCl_3$ or DMSO-d_6. Mass spectra were obtained on a Kratos MS 9/50 instrument. Tlc was on Merck Si gel glass plates. Spots were visualized by short or long wave uv light or by spraying with iodoplatinate spray reagent.

PLANT MATERIAL.—*H. buxbaumii* was collected around the campus of Yarmuk University in Irbid, Jordan, in April 1984. The plant was identified by one of the present authors (D.M. Al-E.), and a voucher specimen was deposited in the Herbarium of the University of Jordan, Amman, Jordan.

EXTRACTION AND CHROMATOGRAPHY.—The dried and ground whole plant material (17 kg) was extracted first with cold petroleum ether and then with cold EtOH. The EtOH extracts were concentrated under reduced pressure, acidified with 5% HCl, and filtered. The filtrate was basified with NH_4OH and extracted with $CHCl_3$. The organic solvent was evaporated, leaving a gummy residue (22 g). This residue was loaded on a Si gel column (70-230 mesh ASTM) (1.4 kg) packed in $CHCl_3$. Elution was with $CHCl_3$ and then with $CHCl_3$ gradually enriched with MeOH. We collected 126 fractions of 250 ml each.

Fractions 7-15 afforded coumarin (200 mg), fraction 24 was mostly justicidin B (150 mg), fractions 25-27 furnished dictamnine (25 mg) and amorphous γ-fagarine (10 mg), fractions 35-43 yielded diphyllin (130 mg), and fractions 90-112 supplied (−)-tuberculatin (4 g). Hydrolysis of (−)-tuberculatin in diluted H_2SO_4 gave rise to diphyllin and the sugar apiose that was identified by comparative tlc and pc with an authentic sample of apiose.

Fractions 53-84 (3.14 g) showed a major band on tlc with a blue fluorescence under uv light. Preparative tlc in the systems toluene-Me_2CO-$CHCl_3$ (40:35:25) and $CHCl_3$-MeOH (90:10) furnished amorphous **2** (1.3 g). Acid hydrolysis of this material supplied diphyllin and apiose.

(−)-MONO-*O*-ACETYL DIPHYLLIN APIOSIDE [**2**].—$C_{28}H_{29}O_{12}$; $[\alpha]_D$ −56.2° (*c* 0.98, MeOH); ir ν max ($CHCl_3$) 1740 cm^{-1}, uv λ max (MeOH) 226, 262, 292, 312, 350 nm (log ε 4.39, 4.73, 4.04, 4.06, 3.72); ms *m/z* 554 (M^+, 0.2%), 380 (100), 351 (3), 320 (7), 293 (14), 217 (8), 175 (7), 139 (27), 97 (18), 43 (59).

(−)-TRI-*O*-ACETYL TUBERCULATIN.—(−)-Tuberculatin [**1**] and **2** were acetylated separately using Ac_2O in pyridine at room temperature. Work-up afforded the triacetate ester, mp 146-149° (MeOH) [lit. (11) mp 145-148°], ν max ($CHCl_3$) 1740 cm^{-1}.

The previously known compounds were identified spectrally (ir, uv, ms, ^{1}H nmr) and by their melting points.

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