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LIGNANS OF *HAPLOPHYLLUM TUBERCULATUM*

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Key Word Index—*Haplophyllum tuberculatum*, Rutaceae, 1-aryl-2,3-naphthalide lignan type A, 4-aryl-2,3-naphthalide lignan type B, diphyllin, justicidin A and B, tuberculatin, triacetyltuberculatin, apiose

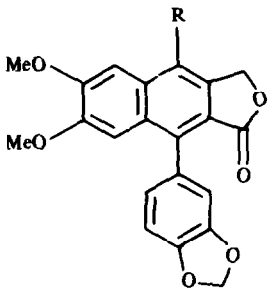
Abstract—Tuberculatin, a new lignan apioside, was isolated from *Haplophyllum tuberculatum* Chemical transformations and spectral evidence established its structure as 4-*O*-(β-D-apiofuranosyl)-6,7-dimethoxy-1-(3′, 4′-methylenedioxyphenyl)-3-hydroxymethylnaphthalene-2-carboxylic acid lactone Three other known 1-aryl-2,3-naphthalide lignans, diphyllin, justicidin A and B occurring with tuberculatin were isolated and characterized

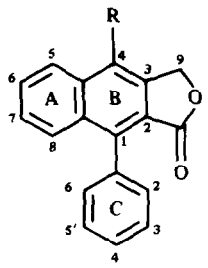
INTRODUCTION

As part of an ongoing phytochemical investigation of folk medicinal plants from Libyan flora, we isolated from *Haplophyllum tuberculatum* (Rutaceae) four lignans of type A (1-aryl-2,3-naphthalide) In this paper we report the isolation and structure elucidation of these four lignans

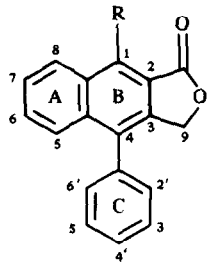
RESULTS AND DISCUSSION

Chromatography (column and TLC) of the chloroform extract of air-dried petrol defatted aerial portions of flowering *Haplophyllum tuberculatum* plants afforded four crystalline lignans The individual spectral (UV, IR, ^{1}H NMR, MS) properties of three of these components (**1**–**3**) were found to correspond closely to those of reported diphyllin (**1**) [1–3], justicidin A (**2**) [1, 2] and justicidin B (**3**) [1, 4] A related fourth constituent was a new lignan apioside for which we proposed the name tuberculatin [4-*O*-(β-D-apiofuranosyl)-6,7-dimethoxy-1-(3′,4′-methylenedioxyphenyl)-3-hydroxymethylnaphthalene-2-carboxylic acid lactone] (**4a**)



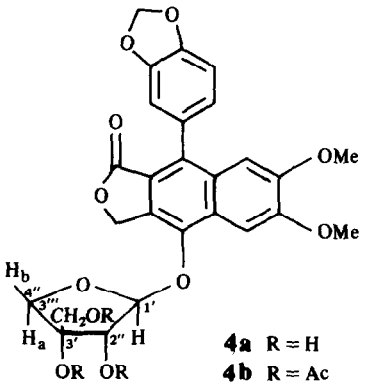


Type – A

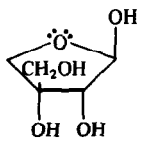


Type – B

1 R = OH
2 R = OMe
3 R = H

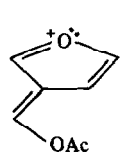


4a R = H
4b R = Ac



5a

5b



5c

The evidence on which we based the assignment of 1-aryl-2,3-naphthalide lignan type A in preference to 4-aryl-2,3-naphthalide type B for the *H tuberculatum* lignans (**1–4a**) was inferred by a comparison of the ^{1}H NMR spectral data with those of reported types A and B lignans (Table 1) [5, 6] It is obvious from the spectral data listed in this table that lignans **1–4a** belong to type A (see [5, 6])

The aromatic proton region of the spectra of lignans **1–4a**, indicates the presence of three proton ABX systems (ring C with either a methylenedioxy or a methoxy pair at C-3′ and C-4′), and two one-proton singlets (ring A with either a methoxy pair or a methylenedioxy at C-6 and C-7), and ring B [R = H, OH, OMe, *O*-apiosyl or *O*-triacetylapiosyl (**4b**)] The spectra also exhibit the absorption of C-6′ proton double-doublets (the more shielded by the naphthalide moiety) centred upfield to the C-2′ proton doublet signal ($\Delta\delta$ 0 03–0 12) and methoxy pair chemical shift difference ($\Delta\delta$ 0 24–0 33 with more shielded 7-methoxy being closer to the influence of the aryl nucleus These findings showed the methylenedioxy to be at the C-3′,4′ position (ring C) and the methoxy pair at the C-6,7 position (ring A) [6, 7] This was unequivocally confirmed by comparison with reported spectra of alternative isomers (types A and B in which a methylenedioxy and a methoxy pair are interchanged) (see refs [4, 6–8])

The assignment of structure **4a** for tuberculatin was based on chemical transformations and spectral (UV, IR, ^{1}H NMR, MS) data The products from acid hydrolysis of tuberculatin were diphyllin **1** (methyl derivative **2**) identified by direct comparison (UV, IR, ^{1}H NMR, MS, TLC, MMP) and apiose, identified by direct TLC comparison with an authentic specimen ([9–11] and colour reactions [12–14])

Tuberculatin and its triacetate derivative, triacetyl-tuberculatin (**4b**), are laevorotatory optically active lignan apiosides The spectral (UV, IR, ^{1}H NMR, MS) data are in agreement with the proposed structures The formulae $C_{26}H_{24}O_{11}$ (M^+ 512 133) of tuberculatin and $C_{32}H_{30}O_{14}$ (M^+ 638 161) of the triacetate were determined from mass spectrometry and elemental analyses The high and low resolution mass spectra of tuberculatin showed the formula $C_{21}H_{16}O_7$ [M^+ 380 090, *m/z* 380 (100%)] of the diphyllin ion moiety **1**, those of triacetyl-tuberculatin showed formulae $C_{21}H_{16}O_7$ [M^+ 380 090, *m/z* 380 (34 34%)] of the diphyllin ion moiety **1**, $C_{11}H_{15}O_7$ [M^+ 259 081, *m/z* 259 (69 34%)] of the triacetylapiosylium ion moiety (**5b**) and $C_7H_7O_3$ [M^+ 139 038, *m/z* 139 (100%)] of its fragment cation (**5c**)

The UV spectra of **4a, b** showed almost the same absorption bands as those of justicidin A (Experimental) Their IR spectra contained bands associated with aromatic γ-lactones (1735 and 1747 cm^{-1}) and methylenedioxy ethers (932 and 934 cm^{-1}) The 400 MHz ^{1}H NMR spectra of **4a, b** exhibited the signals of five aromatic protons with the same substitution pattern as that of justicidin A and diphyllin (Experimental) The assignment of β-configuration for the apiosidic linkage was deduced from ^{1}H NMR spectra The spectra showed the anomeric proton doublet signal of **4a** at δ 5 51, with coupling constant $J_{1'',2''} = 3$ Hz and a dihedral angle $\phi = 126°$, the anomeric proton singlet signal of **4b** absorbed at δ 5 82 with zero coupling constant ($J_{1'',2''} = 0$) and a dihedral angle ($\phi = 90°$) indicating the α-configuration for the anomeric proton and β-apiosidic linkage

The ^{1}H NMR spectra (**1–3, 4b**) in $CDCl_3$ consistently showed the singlet signal ascribable to the C-8 proton at a low field to the doublet signal of the C-5′ proton, but due to the $(CD_3)_2SO$ effect the trend was reversed in **4a**, with the C-5′ proton doublet signal centred at δ 7 06 and the C-8 proton singlet signal occurring at δ 7 03

The non-equivalence of γ-lactone methylene protons of tuberculatin (**4a**) and triacetyl-tuberculatin (**4b**) could be induced by the effect of the β-anomeric chiral centre of the apiosyl and triacetylapiosyl moieties of these lignan molecules and/or by the molecular asymmetry The signals of the double-doublets (*dd*) ascribable to one of the γ-lactone methylene protons (**4a**) resonated at δ 5 50 while that of the other proton was centred at δ 5 54 Hence each proton is split by the other ($J_{gem} = 14$ Hz) and further coupled through seven bonds to the aryl C-2′ proton or the C-6′ proton ($^7J = 1$ Hz), giving rise to double-quartets centred at δ 5 52 ($J = 14, 1$ Hz) However the signals of the other pair of double-doublets associated with the non-equivalent γ-lactone methylene protons (**4b**), displayed at δ 5 44 and 5 52 with coupling constants $J_{gem} = 14$ and $^7J = 1$ Hz, gave rise to double-quartets centred at δ 5 48 ($J = 14, 1$ Hz)

On the basis of the spectral (UV, IR, ^{1}H NMR, MS) data and chemical evidence reported in this paper we assigned structure **4a** to lignan tuberculatin

EXPERIMENTAL

Mps are uncorr UV spectra were recorded on a Beckman Acta MIV spectrometer in $CHCl_3$ soln IR spectra were recorded on a Beckman Acculab 9 spectrometer (KBr disks) Optical rotations

Table 1 Chemical shift values in $CDCl_3$ (except **4a** in $(CD_3)_2SO$ solution)

	Lignans (**1–4a, b**)	Lignans type A (1-aryl-2, 3-naphthalide)*	Lignans Type B (4-aryl-2,3-naphthalide)*
γ-Lactone methylene	5 38–5 55	5 32–5 54	5 08–5 23
H-4	7 71 (3)	*ca* 7 70	
H-1			*ca* 8 30
4-OMe	4 15 (2)	*ca* 4 10	
1-OMe			*ca* 4 35
Ring A (6,7-dimethoxy)	$\Delta\delta$ 0 24–0 33	($\Delta\delta$ 0 21–0 28)	

*See refs [1, 4–8]

(20% MeOH–$CHCl_3$) were measured on a Perkin–Elmer 141 polarimeter ^{1}H NMR spectra were recorded in $CDCl_3$ (for **1–3**, **4b**) in $(CD_3)_2SO$ (for **4a**) solns on a Bucker WH-400 spectrometer, University of British Columbia Chemical shifts are given in δ (ppm) relative to TMS Low resolution MS were recorded on an AEI-MS-902 or Atlas CH-4B spectrometer and high resolution MS on an AEI-MS-902 Instrument, University of British Columbia Microanalyses were carried out by Mr P Borda of the Microanalytical Laboratory, University of British Columbia Silica gel $pF_{254-366}$ (Merck) was used for CC Silica gel D (Riedel) was used for prep chromatography (TLC) with ether as a solvent Al sheets precoated with silica gel 60F254 (Merck) was used for obtaining TLC values for lignans using hexane–EtOAc (1 3) as solvent with visualization under 366 nm UV TLC of sugars was with silica gel 60F254 (Merck) or cellulose F254 (Riedel) in formic acid–methyl ethyl ketone–*t*-BuOH–H_2O (15 30 40 15) [15] as solvent spraying with aniline, diphenylamine and 80% orthophosphoric acid in acetone (1 1 5 50) plus 0 6% benzidine HOAc and heating to 100° for 10 min

Isolation The dried and powdered aerial portions (1 5 kg) of flowering *Haplophyllum tuberculatum* collected in August at Garian (the mountainous area south-west of Tripoli) were extracted successively with petrol (4 × 8 l) and $CHCl_3$ (4 × 8 l) with stirring The petrol extract contained fatty and waxy materials (sitosterol and long chain aliphatic alcohols and ketones), alkaloids (quinoline type) and lignans (justicidin A and B) The $CHCl_3$ (35 g) dissolved in CH_2Cl_2 was absorbed [16] onto silica gel (70 g) and after removal of CH_2Cl_2 the powder was added to a column of silica gel (150 g). Elution was with hexane, hexane–CH_2Cl_2 (19 1) etc up to CH_2Cl_2 then CH_2Cl_2–EtOAc (19 1) etc through to pure EtOAc The lignan bands were detected by UV fluoresence The fast-running zones were those of justicidin A (R_f = 0 42) and B (R_f = 0 39) followed by that of diphyllin (R_f = 0 25) and then the slow-running zone of tuberculatin (R_f = 0 04) The residue of mixed justicidin A and B after several recrystallizations from EtOAc–petrol (40–60°) afforded a crystalline, colourless mixture (*ca* 1 0 g in 1 3 ratio) which was separated into justicidin A (0 20 g) and justicidin B (0 60 g) by twice developed preparative TLC The diphyllin residue after recrystallization from EtOAc and EtOH yielded the pure compound (50 mg) The tuberculatin was recrystallized from EtOAc to give the aposide (*ca* 3 0 g)

Acetylation of tuberculatin was with Ac_2O–pyridine

Acid hydrolysis of tuberculatin with 5% H_2SO_4 at 100° for 4 hr gave diphyllin and apiose (followed by TLC) The diphyllin was extracted by Et_2O and yielded pale yellowish needles (EtOAc and EtOH, 0 15 g) Methylation with diazomethane in Et_2O soln yielded justicidin A The apiose soln was neutralized ($BaCO_3$) evapd to dryness and examined by chromatography (cellulose, silica gel) The characteristic yellow spots of apiose (cellulose R_f = 0 50 and silica R_f = 0 56) showed yellowish white fluorescence in UV after spraying The sugar was identified by co-chromatography (TLC, colour) comparison with authentic apiose

Diphyllin (**1**) Pale yellow needles (50 mg) from EtOH, mp 282–285°, MS, 380 089 (Found C, 65 97, H, 4 22 Calc for $C_{21}H_{16}O_7$ C, 66 30, H, 4 24%, MW, 380 090) ^{1}H NMR ($CDCl_3$), IR (KBr) and UV ($CHCl_3$) as reported in the lit [1–3]

Justicidin A (**2**) Colourless needles (0 20 g) from EtOAc–petrol, mp 260–263°, MS, 394 106 (Found C, 66 75, H, 4 59 Calc for $C_{22}H_{18}O_7$, C, 66 99, H, 4 60%, MW, 394 105) ^{1}H NMR ($CDCl_3$), UV ($CHCl_3$) and IR (KBr) identical with methyl ether of diphyllin in all respects [1, 2]

Justicidin B (**3**) Colourless plates (0 60 g) from EtOAc–petrol, mp 236–238°, MS, 364 093 (Found C, 69 21, H, 4 43 Calc for $C_{21}H_{16}O_6$, C, 69 17, H, 4 43%, MW, 364 095) ^{1}H NMR ($CDCl_3$), UV ($CHCl_3$) and IR (KBr) as reported [1, 4]

Tuberculatin (**4a**) Plates (3 0 g) from EtOAc, mp 245–248°, $[\alpha]_D^{20}$ −107 58° (*c* 1 112, 20% MeOH–$CHCl_3$) MS, 512 133 (Found C, 60 92, H, 4 72 Calc for $C_{26}H_{24}O_{11}$ C, 60 90, H, 4 72%, MW, 512 132) ^{1}H NMR $(CD_3)_2SO$ diphyllin moiety δ 3 80 (3H, *s*, C-7 OMe), 4 13 (3H, *s*, C-6 OMe), 5 52 (2H, *dq*, *J* = 14, 1 Hz, C-9 CH_2), 6 15 (2H, *q*, *J* = 1 Hz, C-3′,4′–OCH_2O–), 6 82 (1 H, *dt*, *J* = 8, 1 Hz, C-6′ H), 6 94 (1H, *t*, *J* = 1 Hz, C-2′ H), 7 06 (1H, *d*, *J* = 8 Hz, C-5′ H), 7 03 (1H, *s*, C-8 H), 7 70 (1H, *s*, C-5 H), apiosyl moiety [17] δ 5 49 (1H, *d*, *J* = 3 Hz, C-1″ H), 4 43 (1H, *dd*, *J* = 6, 3 Hz, C-2″ H), 5 80 (1H, *dd*, *J* = 6, 1 Hz, C-2″ OH), 4 75 (1H, *s*, C-3″ OH), 3 49 (2H, *dq*, *J* = 12, 4 Hz, C-3‴ CH_2), 5 00 (1H, *t*, *J* = 4 Hz, C-3″ OH), 3 81 (1H, *d*, *J* = 10 Hz, C-4″ H_a), 4 26 (1H, *d*, *J* = 10 Hz, C-4″ H_b) UV $\lambda_{max}^{CHCl_3}$ nm (log ε) 263 (4 66), 294 (3 99), 310 (3 99), 352 (3 66) IR ν_{max}^{KBr} cm^{-1} 3510, 1735, 1614, 932, 854, 827, 810

Triacetyltuberculatin (**4b**) Colourless needles (0 20 g) from MeOH, mp 145–148°, $[\alpha]_D^{20}$ −87 12 (*c* 0 521, 20% MeOH–$CHCl_3$), MS, 638 161 (Found C, 59 98, H, 4 72 Calc for $C_{32}H_{30}O_{14}$ C, 60 17, H, 4 74%, MW 638 164) ^{1}H NMR ($CDCl_3$) diphyllin moiety δ 3 80 (3H, *s*, C-7 OMe), 4 13 (3H, *s*, C-6 OMe), 5 48 (2H, *dq*, *J* = 14, 1 Hz, C-9 CH_2), 6 06 (2H, *q*, *J* = 1 Hz, C-3′, 4′–OCH_2O–), 6 78 (1H, *dt*, *J* = 8, 1 Hz, C-6′ H), 6 81 (1H, *t*, *J* = 1 Hz, C-2′ H), 6 94 (1H, *d*, *J* = 8 Hz, C-5′ H), 7 07 (1H, *s*, C-8 H), 7 55 (1H, *s*, C-5 H), triacetylapiosyl moiety δ 5 82 (1H, *s*, C-1″ H), 5 50 (1H, *s*, C-2″ H), 4 94 (2H, *q*, *J* = 12 Hz, C-3‴ CH_2), 4 58 (1H, *d*, *J* = 10 Hz, C-4″ H_a), 4 30 (1H, *d*, *J* = 10 Hz, C-4″ H_b), 2 11, 2 13, 2 17 (3 × 3H, 3*s*, C-2″, 3″, 3‴–OOCMe) UV $\lambda_{max}^{CHCl_3}$ nm (log ε) 263 (4 88), 295 (3 98), 313 (3 77), 352 (3 68) IR ν_{max}^{KBr} cm^{-1} 1747, 1735, 1612, 932, 867, 835, 811

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