[Chem. Pharm. Bull. 30(11)4102—4106(1982)]

Phloroglucinol Derivatives of *Dryopteris abbreviata*[1]

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(Received May 24, 1982)

The phloroglucinol derivatives of *Dryopteris abbreviata* (DC.) NEWMAN from Turkey, a member of the European *D. filix-mas* complex, were investigated.

A known compound, flavaspidic acid PB (I), and three new compounds, designated as dimethylphlorobutyrophenone (II), abbreviatin PB (III) and trisabbreviatin BBB (IV), were isolated in addition to the already reported compounds, filixic acid, flavaspidic acid AB and abbreviatin BB (V). This is the first report of the occurrence of a series of phloroglucinol derivatives lacking the filicinic acid ring moiety, *i.e.*, compounds II, III, IV and V, in European taxa of the *D. filix-mas* complex.

Keywords—*Dryopteris abbreviata*; Aspidiaceae; fern; phloroglucinol derivatives; flavaspidic acid PB; dimethylphlorobutyrophenone; abbreviatin PB; trisabbreviatin BBB

Dryopteris abbreviata (DC.) NEWMAN is a member of the European *D. filix-mas* complex. This diploid species is abundant in the north-eastern part of Turkey, and also occurs in Central Europe.[3,4] The ferns of these taxa in general contain various amounts of filixic acid and a large amount of flavaspidic acid, and *para*-aspidin is usually present in small amounts.[5] Widén *et al.* examined the rhizomes of this species from Scotland and Italy and reported the occurrence of flavaspidic acid and filixic acid.[6] Tanker and Coşkun detected the presence of *para*-aspidin and some unknown compounds in the rhizomes of *D. abbreviata* collected in Turkey.[7] In our previous report[1] concerning the phloroglucinol derivatives from *D. abbreviata*, the isolation and structural elucidation of abbreviatin BB were described.

This paper deals with the isolation of further compounds and their identification. The dried rhizomes of *D. abbreviata* were percolated with ether. The ether extract treated by the magnesium oxide method[8] afforded crude filicin, which consists of a mixture of phloroglucinol derivatives. The isolation of phlorogliucnol derivatives from the crude filicin was carried out as described in "Experimental". The crude filicin yielded a known compound, flavaspidic acid PB (I), and three new compounds, designated as dimethylphlorobutyrophenone (II), abbreviatin PB (III) and trisabbreviatin BBB (IV), in addition to filixic acid, flavaspidic acid AB and abbreviatin BB (V).[1,6]

I was recrystallized from *n*-hexane to give a pale yellow powder. The melting point and spectral data of I were in good agreement with those of authentic flavaspidic acid PB. This is the first reported isolation of I from *D. abbreviata*.

II was recrystallized from cyclohexane to give yellow needles, $C_{12}H_{16}O_4$, mp 135—137°C, showing *Rf* 0.29 on thin layer chromatography (TLC), for which precoated reversed-phase TLC plates (RP-$8_{F\ 254}$) were used, with tetrahydrofuran–phosphoric acid–water (65 : 0.1 : 45) for development. II gave a yellow spot on TLC when sprayed with aqueous tetrazotized di-*O*-anisidine (Fast Blue Salt B) solution. The infrared (IR) spectrum of II showed absorption bands at 3500 (OH), 2930 (CH), 1600 (C=O) and 1570 (C=C) cm^{-1}. The ultraviolet (UV) spectrum of II showed absorption maxima at 222, 293 and 335 nm. The bathochromic shift of the absorption maximum in the presence of a base indicated the presence of phenolic hydroxyl groups. The behavior of the UV spectra resembled that of the sepctra of V, suggesting II to be a phloroglucinol derivative. The proton nuclear magnetic resonance (^{1}H-NMR)

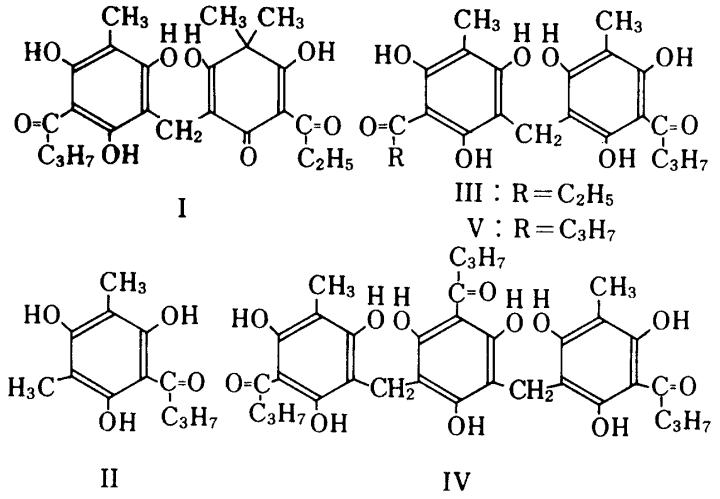

Chart 1

spectrum of II gave signals due to a butyryl and two aromatic methyl groups. The mass spectrum (MS) of II gave significant peaks at *m/z* 224 (M^+, $C_{12}H_{16}O_4$) and 181 ($C_9H_9O_4^+$). The properties of II were in good agreement with those of dimethylphlorobutyrophenone in the literature.[9] Thus, the structure of II was elucidated to be dimethylphlorobutyrophenone, so far known only as a synthetic product.

III was recrystallized from chloroform to give a pale yellow powder, $C_{22}H_{26}O_8$, mp 206—208°C. The IR spectrum of III showed absorption bands at 3400 (OH), 2950 (CH), 1610 (C=O) and 1570 (C=C) cm^{-1}. The UV spectrum of III was very similar to that of V, suggesting III to be an abbreviatin homologue. The ^{1}H-NMR spectrum of III gave signals due to a butyryl, a propionyl and two aromatic methyl groups and a methylene bridge between two aromatic rings. The MS of III gave significant peaks, as shown in Chart 2, whose fragmentations

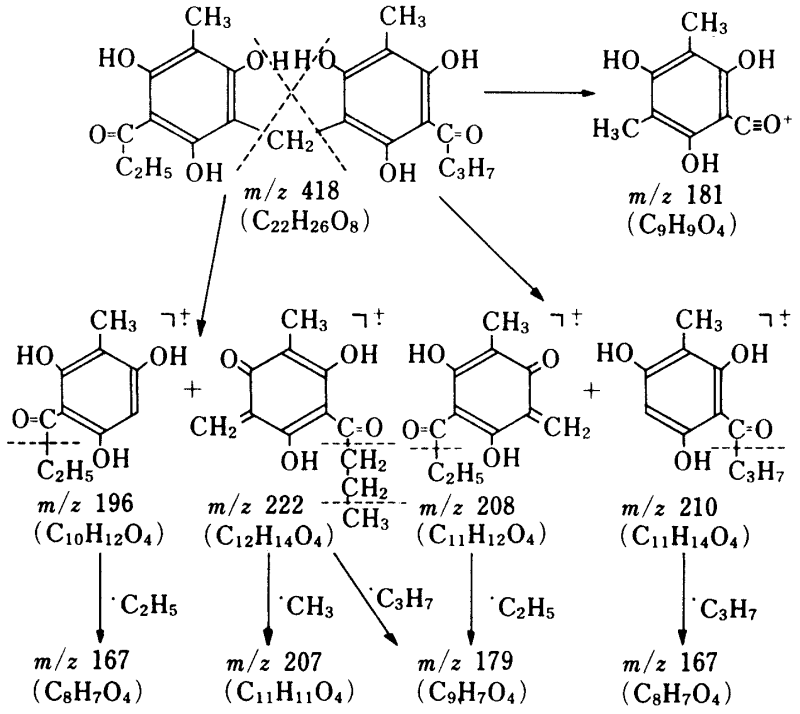

Chart 2. The Mass Spectral Fragmentation of Abbreviatin PB

supported the presence of a butyryl group and a propionyl group on the methylene-bis-methylphloroglucinol skeleton. Consequently, abbreviatin PB was assigned the structure III. III is a new phloroglucinol derivative and the second occurrence of abbreviatin homologues in *Dryopteris* ferns.

IV was recrystallized from cyclohexane–ethanol to give a pale yellow powder, $C_{34}H_{40}O_{12}$, whose molecular weight was confirmed by the observation of *m/z* 640 (M^+) on field desorption mass spectrometry (FD-MS). The IR spectrum of IV showed absorption bands at 3350 (OH), 2900 (CH), 1605 (C=O) and 1570 (C=C) cm^{-1}. The UV spectrum of IV showed absorption maxima at 227, 298 and 330 nm, and an absorption maximum at 328 nm appeared on the addition of base.

The above data indicate that IV has phenolic hydroxyl groups and lacks the filicinic acid ring moiety. The ^{1}H-NMR spectrum of IV was correlated with that of V (Table I). In particular, the appearance of a single signal at δ 4.28 due to two methylene groups in the ^{1}H-NMR spectrum suggested that IV has three aromatic rings linked with each other by methylene

Table I. ^{1}H-NMR Spectral Data for IV and V[a]

	$CH_3CH_2CH_2CO$–			CH_3–	–CH_2–
IV	0.95 (6H, t) J=7.5 Hz	1.83 (4H, m)	3.42 (4H, t) J=7.5 Hz	2.45 (6H, s)	4.28 (4H, s)
	0.90 (3H, t) J=7.5 Hz	1.82 (2H, m)	3.22 (2H, t) J=7.5 Hz		
V	0.93 (0.99 (6H, t) J=7.5 Hz	1.80 1.73 (4H, m)	3.27 3.08 (4H, t) J=7.5 Hz	2.36 2.07 (6H, s)	4.07 3.80)[b] (2H, s)

a) The spectra were taken in pyridine-d_5.
b) The spectrum was taken in $CDCl_3$.

bridges and that the ends are symmetrical, carrying the same acylphloroglucinol group. This view was supported by comparisons of the chemical shifts due to methylene groups in the ^{1}H-NMR spectra (in deuterochloroform)[10] of methylene-bis-aspidinol (δ 3.70), margaspidin BB (δ 3.75), aemulin BB (δ 3.83) and trisaemulin BAB (δ 3.84 and 3.72), whose structures are similar to those of IV and V. On the basis of these data and its coexistence with III and V, IV was proposed to have a structure composed of methylene-bis-methylphlorobutyrophenone and methylphlorobutyrophenone moieties.

This is the first report of the occurrence of a series of phloroglucinol derivatives lacking the filicinic acid ring moiety in European taxa of the *D. filix-mas* complex. It is interesting to note the occurrence of similar phloroglucinol derivatives from *D. aemula* belonging to European taxa of the *D. dilatata* complex.[5,10]

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. The following instruments were used: IR spectra, Shimadzu IR-400; UV spectra, Shimadzu UV-210; ^{1}H-NMR spectra, Hitachi R-40 (90 MHz) and JEOL JNM-FX 200 (200 MHz) with tetramethylsilane (δ=0) as an internal reference; MS, Hitachi RMU-7L and Shimadzu LKB-9000; FD-MS, JEOL JMS-DX 300.

Precoated TLC plates, RP-8_{F254} (Merck), were used for TLC and developed with tetrahydrofuran (THF)–phosphoric acid–water (65: 0.1: 45). The spots were detected by spraying with 0.1% aqueous tetrazotized di-*O*-anisidine (Fast Blue Salt B) solution. When color development was very poor, the color was intensified by overspraying 0.1 N NaOH solution. High performance liquid chromatography (HPLC) was run on a

Shimadzu 841 instrument equipped with a 150×4 mm ID Nucleosil-5C_{18} column. The UV detector was equipped with a 254 nm filter. A THF solution of the sample (4 μl) was injected, and THF-phosphoric acid–water (65: 0.1: 45) was used as the solvent system. The flow-rate was 0.4 ml/min, and the pressure drop 140 kg/cm^2. Silica gel (100 mesh, Mallinckrodt) was used for column chromatography.

The abbreviations used are as follows: s, singlet; t, triplet; q, quartet; m, multiplet; br, broad; sh, shoulder.

Plant Material——*Dryopteris abbreviata* (DC.) Newman was collected on 2 June, 1981, near Trabzon, Turkey. A voucher specimen is retained in Ankara Üniversitesi Eczacilik Fakültesi Herbaryumu (AEF).

Isolation——Dry powdered rhizomes (1.5 kg) were percolated with Et_2O (41 each×3 times). The ethereal solution was evaporated to dryness *in vacuo* to give 148 g of dark green oil. A portion of the extract (100 g) was treated as described in a previous paper,[8] giving 23 g of crude filicin. The crude filicin was dissolved in the least possible quantity of Et_2O and allowed to stand overnight in a refrigerator to yield 800 mg of flavaspidic acid AB. After the removal of flavaspidic acid AB, the mother liquor was chromatographed on 180 g of silica gel using a cyclohexane–THF gradient system (19: 1 for frs. 1—68, 9: 1 for frs. 69—168 and 4: 1 for frs. 169—207). The fractions (10 ml each) were monitored by TLC and HPLC. Fractions 1 to 9 gave 100 mg of crystals. The crystals were shown to be filixic acid, consisting of filixic acid ABA, ABB and BBB, by TLC and HPLC (TLC: *Rf* 0.20, 0.14, 0.10. HPLC: t_R 5.5, 6.5, 8.3 min). Fractions 10 to 16 were subjected to column chromatography over silica gel with a cyclohexane–$CHCl_3$ gradient (90: 2 and 90: 10). Fraction 6 from rechromatography gave 21 mg of flavaspidic acid PB (I) on recrystallization from *n*-hexane. Fractions 17 to 45 gave 350 mg of flavaspidic acid on recrystallization from benzene, and this product was shown to consist of flavaspidic acid AB, PB and BB by TLC (*Rf* 0.46, 0.42, 0.38). Fractions 46 to 68 gave 3 mg of trisabbreviatin BBB (IV) on recrystallization from cyclohexane–ethanol. On fractional recrystallization, fractions 69 to 128 gave first 150 mg of dimethylphlorobutyrophenone (II) from cyclohexane and then 60 mg of abbreviatin BB (V) from $CHCl_3$. Fractions 129 to 132 gave 2 mg of abbreviatin PB (III) on recrystallization from $CHCl_3$. Fractions 133 to 207 gave no crystals.

Flavaspidic Acid PB (I)——Pale yellow powder, mp 156—158°C. TLC: *Rf* 0.42. HPLC: t_R 4.22 min. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2910 (CH), 1640 (C=O), 1610 (C=C), 1470, 1195, 1140 (C–O). UV λ_{max}^{EtOH} nm (log ε): 224 (4.46), 299 (4.42), 349 (4.26). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 307. ^{1}H-NMR (in $CDCl_3$) δ: 0.99 (3H, t, J=7.5 Hz, $-COCH_2CH_2C\underline{H}_3$), 1.14 (3H, t, J=7.5 Hz, $-COCH_2C\underline{H}_3$), 1.48 (6H, s, *gem*-dimethyl), 1.83 (2H, m, $-COCH_2-C\underline{H}_2CH_3$), 2.07 (3H, s, aromatic-CH_3), 3.08 (2H, t, J=7.5 Hz, $-COC\underline{H}_2CH_2CH_3$), 3.17 (2H, q, J=7.5 Hz, $-COC\underline{H}_2CH_3$), 3.50 (2H, s, =C–CH_2–C=), 6.00 (2H, br, OH), 15.70 (2H, br, OH), 18.40 (1H, s, OH). MS *m/z*: 432 (M^+, $C_{23}H_{28}O_8$), 167 ($C_8H_7O_4^+$).

The compound was identical with authentic flavaspidic acid PB.

Dimethylphlorobutyrophenone (II)——Yellow needles, mp 135—137°C (lit.[9] mp 140°C). TLC: *Rf* 0.29. HPLC: t_R 6.97 min. IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 2930 (CH), 1600 (C=O), 1570 (C=C), 1120 (C–O). UV λ_{max}^{EtOH} nm (log ε): 222 (4.17), 293 (4.25), 335 (3.39). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 336. MS: Calcd for $C_{12}H_{16}O_4$, 224.1046. Obsd., 224.1037. ^{1}H-NMR (in $CDCl_3$) δ: 0.95 (3H, t, J=7.5 Hz, $-COCH_2CH_2C\underline{H}_3$), 1.68 (2H, m, $-COCH_2-C\underline{H}_2CH_3$), 2.04 (6H, s, two aromatic-CH_3), 3.04 (2H, t, J=7.5 Hz, $-COC\underline{H}_2CH_2CH_3$), 5.25 (1H, s, OH), 9.55 (2H, s, OH). MS *m/z*: 224 (M^+, $C_{12}H_6O_4$), 181 ($C_9H_9O_4^+$).

The properties of II were in good agreement with those of dimethylphlorobutyrophenone given in the literature.[9]

Abbreviatin PB (III)——Pale yellow powder, mp 206—208°C. TLC: *Rf* 0.23. HPLC: t_R 14.04 min. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2950 (CH), 1610 (C=O), 1570 (C=C), 1460, 1140, 1110 (C–O). UV λ_{max}^{EtOH} nm (log ε): 229 (4.42), 295 (4.41), 329 (4.24). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 333. MS: Calcd for $C_{22}H_{26}O_8$, 418.1637. Obsd., 418.1638. ^{1}H-NMR (in $CDCl_3$) δ: 0.99 (3H, t, J=7.5 Hz, $-COCH_2CH_2C\underline{H}_3$), 1.19 (3H, t, J=7.5 Hz, $-COCH_2-C\underline{H}_3$), *ca.* 1.73 (2H, m, $-COCH_2C\underline{H}_2CH_3$), 2.07 (6H, s, two aromatic-CH_3), 3.08 (2H, t, J=7.5 Hz, $-COC\underline{H}_2-CH_2CH_3$), 3.10 (2H, q, J=7.5 Hz, $-COC\underline{H}_2CH_3$), 3.80 (2H, s, =C–CH_2–C=), MS *m/z*: 418 (M^+), 222, 210, 208, 207, 196, 181, 179, 167.

Trisabbreviatin BBB (IV)——Pale yellow powder. TLC: *Rf* 0.35. HPLC: t_R 10.32 min. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 2900 (CH), 1605 (C=O), 1570 (C=C), 1430, 1380, 1160 (C–O). UV λ_{max}^{EtOH} nm: 227, 298, 330 (sh). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 328. FD-MS *m/z*: 640 (M^+, $C_{34}H_{40}O_{12}$), 432 ($C_{23}H_{28}O_8^+$).

Acknowledgement We wish to thank Asst. Prof. S. Yamanouchi, Department of Pharmacy, College of Sciences and Technology, Nihon University, for measurement of MS with a Hitachi RMU-7L spectrometer and the staff of JEOL Ltd. for measurements of FD-MS spectra with a JEOL JMS-DX 300 spectrometer and ^{1}H-NMR spectra with a JEOL JNM-FX 200 spectrometer.

References and Notes

1) Part II in the series "Phloroglucinol Derivatives of *Dryopteris abbreviata*." For Part I see M. Coşkun, A. Sakushima, S. Nishibe, S. Hisada, and N. Tanker, *Phytochemistry*, **21**, 1453 (1982).
2) Present address: *Department of Pharmaceutical Botany, Faculty of Pharmacy, University of Ankara,*

Ankara, Turkey.
3) N. Tanker and M. Coşkun, *J. Fac. Pharm. Ankara*, **8**, 114 (1978).
4) P.H. Davis, "Flora of Turkey and the East Aegean Islands I," Robert Cunnigan and Sons Ltd., Edinburgh, 1965.
5) C.-J. Widén, *Farm. Aikakausl.*, **81**, 91 (1972).
6) C.-J. Widén, G. Vida, J. Euw, and T. Reichstein, *Helv. Chim. Acta*, **54**, 2824 (1971).
7) N. Tanker and M. Coşkun, *J. Fac. Pharm. Ankara*, **8**, 1 (1978).
8) S. Hisada, S. Nishibe, O. Inoue, I. Inagaki, and Y. Ogihara, *Shoyakugaku Zasshi*, **34**, 8 (1980).
9) P. Karrer, *Helv. Chim. Acta*, **2**, 466 (1919).
10) C.-J. Widén, M. Lounasmaa, G. Vida, and T. Reichstein, *Helv. Chim. Acta*, **58**, 880 (1975).