[Chem. Pharm. Bull. 32(8)3267—3270(1984)]

Studies on the Constituents of *Hedysarum polybotrys* HAND.-MAZZ.

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(Received December 16, 1983)

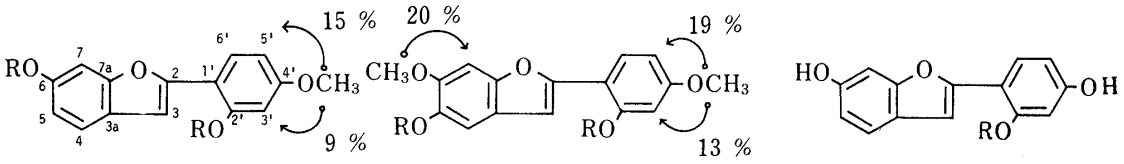
Thirteen phenolic compounds were isolated from *Hedysarum polybotrys* HAND.-MAZZ., and their structures were determined by spectroscopic studies. Among the thirteen, 5-hydroxy-2-(2-hydroxy-4-methoxyphenyl)-6-methoxybenzofuran (X) is a new compound, and 6-hydroxy-2-(2-hydroxy-4-methoxyphenyl)-benzofuran (VIII) was isolated from this plant for the first time.

Keywords——Leguminosae; *Hedysarum polybotrys*; phenolic compounds; benzofuran

In connection with our studies on the components of some Leguminosae, we also investigated *Hedysarum polybotrys* HAND.-MAZZ. (−)-3-Hydroxy-9-methoxypterocarpan (II) has been isolated as an antibacterial substance[1] and some saponins were reported as constituents[2] of this plant.

A new benzofuran, 5-hydroxy-2-(2-hydroxy-4-methoxyphenyl)-6-methoxybenzofuran (X) was isolated from the ethyl acetate-soluble fraction of a methanolic extract of the root, together with formononetin (I), (−)-3-hydroxy-9-methoxypterocarpan (II), alkyl ferulate (III), afromosin (IV), liquiritigenin (V), isoliquiritigenin (VI), (−)-vestitol (VII), 3′,7-dihydroxy-4′-methoxyisoflavone (IX), vanillic acid (XI), 3,4,5-trimethoxycinnamic acid methyl ester (XII) and 6-hydroxy-2-(2-hydroxy-4-methoxyphenyl)-benzofuran (VIII), and ononin (XIII) was isolated from the *n*-butanol-soluble fraction of the methanolic extract. This is the first time that VIII has been obtained from the plant, but it has been synthesized in connection with work on a phytoalexin.[3] These known compounds were identified by direct comparison with authentic samples or by comparison of spectral and physical data with literature value.

5-Hydroxy-2-(2-hydroxy-4-methoxyphenyl)-6-methoxybenzofuran (X) was isolated as the diacetate (Xa), $C_{20}H_{18}O_7$, M^+ 370.1085 (Calcd for $C_{20}H_{18}O_7$ 370.1055), mp 164—168 °C, colorless needles. Its ultraviolet (UV) spectrum showed absorption maxima at 280 (4.16), 289 (sh 4.19), 302 (sh 4.35), 315 (4.54) and 329 (4.46), suggesting X to be a 2-phenylbenzofuran derivative.[4] The proton magnetic resonance (^{1}H-NMR) spectrum of Xa showed two acetyl signals at δ 2.34 and 2.40 and two methoxyl signals at δ 3.84 and 3.88. Furthermore, in the olefinic proton region ABX-type proton signals at δ 6.69 (1H, d, J=2.5 Hz), 6.87 (1H, dd, J=9, 2.5 Hz), 7.81 (1H, d, J=9 Hz) attributed to H-3′, H-5′ and H-6′, respectively, and three signals at δ 6.78 (1H, d, J=1 Hz), 7.09 (1H, br s), 7.19 (1H, s) were observed. The two signals at δ 6.78 and 7.07 showed long-range coupling with each other. In general, zig-zag coupling is observed between H-3 and H-7 in benzofuran derivatives.[5] Therefore these three signals were assigned to H-3, H-7 and H-4, respectively. The locations of the methoxyl groups were confirmed by the observation of nuclear Overhauser effect (NOE) at H-7 (20%), H-3′ (13%) and H-5′ (19%) and by the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectroscopic signals in comparison with those of 2-(2,4-dihydroxyphenyl)-6-hydroxybenzofuran (XIV),[4] 6-demethylvignafuran (XV)[3] and VIII. From these data, the structure of X was established as 5-hydroxy-2-(2-hydroxy-4-methoxyphenyl)-6-methoxybenzofuran.



VIII : R=H
VIIIa : R=$COCH_3$

X : R=H
Xa : R=$COCH_3$

XIV : R=H
XV : R=CH_3

Chart 1

TABLE I. ^{13}C Chemical Shifts of XIV, XV, VIII, X and Xa in Acetone-d_6

Carbon No.	XIV	XV	VIII	X	Xa
2	153.0	152.2	152.7	153.0	152.5[c]
3	103.9[a]	104.3	104.4	104.3	103.5
3a	123.4	123.2	123.4	123.3	121.7
4	121.4	121.2	121.5	105.5	112.4[d]
5	112.5	112.5	112.7	144.3[b]	137.1
6	156.0	155.8	156.1	146.6[b]	148.2
7	98.3	98.1	98.3	95.7	95.8
7a	155.3	155.4	155.7	148.6	151.9[c]
1′	111.0	108.3	112.2	112.1	116.4
2′	155.5	159.2	156.1	155.8	149.5
3′	104.0[a]	100.2	102.9	102.8	109.2
4′	158.8	158.4	161.5	161.2	160.3
5′	108.4	108.3	106.5	106.4	113.9[d]
6′	128.1	127.8	127.9	127.7	128.5
				55.5	55.6
OCH_3		55.6	55.6	56.8	56.4
					20.6
$OCOCH_3$					21.3

a—d) Assignments may be interchanged in each column.

Experimental

Melting points were determined on a Yanaco MP-500 micromelting point apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-140 digital polarimeter. Infrared (IR) spectra were run on a JASCO IRA-2 grating infrared spectrophotometer and UV spectra on a Shimadzu UV-360 recording spectrophotometer. Mass spectra (MS) were recorded on a JEOL JMS-D/100 and high-resolution MS on a JMS-O/SG-2 mass spectrometer. ^1H-NMR and ^{13}C-NMR spectra were recorded on a JEOL FX-90 Q machine (89.55 and 22.5 MHz, respectively). Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; br, broad). Gas chromatography (GC) was run on a Hitachi K 53 gas chromatograph.

Isolation——Dried roots of *Hedysarum polybotrys* (7.5 kg) were extracted with methanol under reflux. The extract was concentrated *in vacuo* and the residue was suspended in water. This suspension was extracted with ethyl acetate and *n*-butanol, successively. The ethyl acetate-soluble fraction was concentrated *in vacuo* to afford a residue (100 g), which afforded I—XII after repeated chromatography on silica gel. The *n*-butanol-soluble fraction was concentrated *in vacuo* to afford a residue (58 g), which afforded XIII after chromatography on silica gel.

Formononetin (I)——Colorless plates (2 g), mp 257—265 °C (methanol) (lit. mp 264—265 °C).[6] IR ν_{max}^{KBr} cm^{-1}: 2930, 1640, 1605, 1595, 1515, 1450, 1270, 1245, 1175, 1025, 805. ^1H-NMR (pyridine-d_5) δ: 3.70 (3H, s, OCH_3), 7.07 (2H, d, J=9 Hz, H-3′, H-5′), 7.09 (1H, d, J=2 Hz, H-8), 7.18 (1H, dd, J=9, 2 Hz, H-6), 7.78 (2H, d, J=9 Hz, H-2′, H-6′), 8.13 (1H, s, H-2), 8.42 (1H, d, J=9 Hz, H-5).

(−)-3-Hydroxy-9-methoxypterocarpan (II)——Colorless crystals (1.7 g), mp 132—140 °C (benzene) (lit. mp 131—132 °C).[1] $[\alpha]_D^{24}$ −220.6° (c=0.75, chloroform). IR ν_{max}^{KBr} cm^{-1}: 3400, 1620, 1590, 1495, 1470, 1150, 950, 825. ^1H-

NMR (acetone-d_6) δ: 3.58 (2H, m, H-6ax, H-6a), 3.74 (3H, s, OCH_3), 4.26 (1H, dd, J=10, 8 Hz, H-6 eq), 5.50 (1H, d, J=6 Hz, H-11a), 6.37 (2H, br s, H-4, H-10), 6.41 (1H, dd, J=9, 2 Hz, H-8), 6.55 (1H, dd, J=8, 2 Hz, H-2), 7.21 (1H, d, J=9 Hz, H-7), 7.31 (1H, d, J=8 Hz, H-1).

Alkyl Ferulate (III)——White waxy solid (1 g). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540, 2920, 2860, 1700, 1630, 1605, 1590, 1510, 1465, 1430, 1260, 1170, 1120, 1030, 980, 845, 820. ^{1}H-NMR (acetone-d_6) δ: 0.89 (3H, s, CH_3), 1.28 (br s, $(-CH_2-)_n$), 3.89 (3H, s, OCH_3), 4.15 (2H, t, J=7 Hz, OCH_2), 5.32 (m, CH=CH), 6.33 (1H, d, J=16 Hz, CO–CH=C), 6.84 (1H, d, J=8 Hz, H-5), 7.08 (1H, dd, J=8, 2 Hz, H-6), 7.22 (1H, d, J=2 Hz, H-2), 7.58 (1H, d, J=16 Hz, CO–C=CH). ^{13}C-NMR (acetone-d_6) δ: 14.2 (CH_3), 23.1, 26.0, 26.5, 27.6, 28.0, 28.8, 29.4, 29.9, 30.2, 30.5, 31.4, 32.0, 32.4, 56.1 (OCH_3), 64.1 (OCH_2), 111.0 (C-2), 115.6; 115.7 (CO–C=C, C-5), 123.4 (C-6), 128.5; 130.4 (C=C), 145.2 (CO–C=C), 148.3 (C-4), 149.7 (C-3), 167.2 (C=O).[7]

Afromosin (IV)——Colorless needles (20 mg), mp 229—237 °C (methanol) (lit. mp 228—229 °C).[8] IR ν_{max}^{KBr} cm^{-1}: 3430, 1620, 1565, 1515, 1275, 1245, 835. ^{1}H-NMR (pyridine-d_5) δ: 3.90 (3H, s, OCH_3 at C-4′), 3.95 (3H, s, OCH_3 at C-6), 7.05 (2H, d, J=9 Hz, H-3′, H-5′), 7.17 (1H, s, H-8), 7.76 (2H, d, J=9 Hz, H-2′, H-6′), 7.86 (1H, s, H-5), 8.12 (1H, s, H-2).

Liquiritigenin (V)——Colorless needles (50 mg), mp 204—206 °C (dec.) (methanol) (lit. mp 207—208 °C).[9] IR ν_{max}^{KBr} cm^{-1}: 3250, 1655, 1600, 1520, 1465, 1325, 1250, 1230, 1215, 1160, 1115, 830. ^{1}H-NMR (acetone-d_6) δ: 2.66 (1H, dd, J=17, 4 Hz, H-3β), 3.00 (1H, dd, J=17, 13 Hz, H-3α), 5.45 (1H, dd, J=13, 4 Hz, H-2), 6.21 (1H, d, J=2 Hz, H-8), 6.55 (1H, dd, J=9, 2 Hz, H-6), 6.89 (2H, d, J=9 Hz, H-3′, H-5′), 7.39 (2H, d, J=9 Hz, H-2′, H-6′), 7.72 (1H, d, J=9 Hz, H-5).

Isoliquiritigenin (VI)——Yellow needles (150 mg), mp 199—201 °C (methanol–water) (synthetic product mp 202—204 °C).[9] IR ν_{max}^{KBr} cm^{-1}: 3400, 3300, 1630, 1605, 1585, 1550, 1515, 1365, 1215, 1160. ^{1}H-NMR (acetone-d_6) δ: 6.36 (1H, d, J=2 Hz, H-3′), 6.43 (1H, dd, J=9, 2 Hz, H-5′), 6.92 (2H, d, J=8 Hz, H-3, H-5), 7.72 (2H, d, J=9 Hz, H-2, H-6), 7.77 (2H, s, H-α, H-β), 8.09 (1H, d, J=9 Hz, H-6′).

(−)-Vestitol (VII)——Colorless needles (120 mg), mp 159—160 °C (acetone–benzene) (lit. mp 156 °C).[10] $[\alpha]_D^{18}$ −35.1° (c=0.88, methanol). IR ν_{max}^{KBr} cm^{-1}: 3350, 1620, 1590, 1525, 1505, 1450, 1430, 1310, 1220, 1200, 1155, 1135, 1115, 1030, 843, 825, 790. ^{1}H-NMR (acetone-d_6) δ: 3.72 (3H, s, OCH_3), 6.28 (1H, br s, H-3′), 6.32 (1H, dd, J=8, 2 Hz, H-5′), 6.45 (1H, dd, J=8, 2 Hz, H-6), 6.47 (1H, br s, H-8), 6.88 (1H, d, J=8 Hz, H-6′), 7.04 (1H, d, J=8 Hz, H-5).

6-Hydroxy-2-(2-hydroxy-4-methoxyphenyl)-benzofuran (VIII)——Colorless needles (15 mg), mp 172—173 °C (methanol–benzene). UV λ_{max}^{MeOH} (log ε): 334.5 (4.57), 320 (4.59), 3.06 (sh 4.34), 281 (4.15), 273 (sh 4.11). IR ν_{max}^{KBr} cm^{-1}: 3450, 3320, 1610, 1585, 1508, 1483, 1290, 1205, 1160, 1140, 1110, 1030, 830. MS m/z: 256.0775 (Calcd for $C_{15}H_{12}O_4$: 256.0738), 241.0523 (Calcd for $C_{14}H_9O_4$: 241.0502). ^{1}H-NMR (acetone-d_6) δ: 3.80 (3H, s, OCH_3), 6.53 (1H, dd, J=9, 2.5 Hz, H-5′), 6.62 (1H, d, J=2.5 Hz, H-3′), 6.79 (1H, dd, J=8, 2 Hz, H-5), 6.99 (1H, m, H-7), 7.19 (1H, d, J=1 Hz, H-3), 7.38 (1H, d, J=8 Hz, H-4), 7.80 (1H, d, J=9 Hz, H-6′). ^{13}C-NMR (acetone-d_6) δ: Table I.[3]

7,3′-Dihydroxy-4′-methoxyisoflavone (IX)——Colorless needles (10 mg), mp 253—257 °C (methanol–chloroform) (lit. mp 251—253 °C).[6] IR ν_{max}^{KBr} cm^{-1}: 3420, 3160, 1620, 1570, 1510, 1280, 1240, 850. ^{1}H-NMR (pyridine-d_5) δ: 3.77 (3H, s, OCH_3), 7.04 (1H, d, J=9 Hz, H-5′), 7.08 (1H, d, J=2 Hz, H-8), 7.17 (1H, dd, J=9, 2 Hz, H-6), 7.30 (1H, dd, J=9, 2 Hz, H-6′), 7.77 (1H, d, J=2 Hz, H-2′), 8.15 (1H, s, H-2), 8.41 (1H, d, J=9 Hz, H-5).

Vanillic Acid (XI)——Colorless needles (120 mg), mp 203—207 °C (methanol–water) (synthetic product mp 205 °C).[11] IR ν_{max}^{KBr} cm^{-1}: 3480, 1675, 1595, 1520, 1430, 1295, 1275, 1235, 1200, 1025, 755. ^{1}H-NMR (acetone-d_6) δ: 3.92 (3H, s, OCH_3), 6.90 (1H, d, J=9 Hz, H-5), 7.55 (1H, br s, H-2), 7.59 (1H, dd, J=9, 2 Hz, H-6).

3,4,5-Trimethoxycinnamic Acid Methyl Ester (XII)——Colorless needles (110 mg), mp 99—100 °C (methanol) (synthetic product mp 91—91.5 °C).[12] IR ν_{max}^{KBr} cm^{-1}: 1690, 1630, 1575, 1500, 1460, 1415, 1335, 1285, 1245, 1120, 1000, 975, 815. ^{1}H-NMR ($CDCl_3$) δ: 3.81 (3H, s, $COOCH_3$), 3.89 (9H, s, $OCH_3 \times 3$), 6.33 (1H, d, J=16 Hz, CO–CH=C), 6.74 (2H, s, H-2, H-6), 7.61 (1H, d, J=16 Hz, CO–C=CH).

Ononin (XIII)——Colorless prisms (210 mg), mp 214—216 °C (dec.) (methanol) (lit. mp 223 °C).[6] IR ν_{max}^{KBr} cm^{-1}: 3400, 1665, 1600, 1250, 1170, 1110. ^{1}H-NMR (pyridine-d_5) δ: 3.71 (3H, s, OCH_3), 5.75 (1H, d, J=7 Hz, anomeric H), 7.06 (2H, d, J=9 Hz, H-3′, H-5′), 7.21 (1H, br s, H-8), 7.36 (1H, dd, J=9, 2 Hz, H-6), 7.74 (2H, d, J=9 Hz, H-2′, H-6′), 8.11 (1H, s, H-2), 8.33 (1H, d, J=9 Hz, H-5).

Saponofication of III——III (45 mg) was dissolved in a mixture of dioxane (2 ml) and 2% sodium hydroxide (2 ml) and the whole was stirred for 4 h at room temperature under a nitrogen atmosphere. The reaction mixture was diluted with water and extracted 3 times with ether, then the water layer was extracted with ethyl acetate 3 times after acidification with dilute hydrogen chloride. The ether extract was washed with water and dried over sodium sulfate and, then the ether was evaporated off to give the alcohol (IIIa) (22 mg) as a colorless oil. IIIa was oxidized with CrO_3–pyridine complex and methylated with diazomethane in the usual manner and then identified by GC as a mixture (*ca.* 1 : 1) of cetyl alcohol and oleyl alcohol. Conditions : column, 8% Igepal, 3 mm × 1 m; column temperature 200 °C; carrier gas, N_2; t_R 4.6 min (methyl palmitate), 10.0 min (methyl oleate). The ethyl acetate extract was washed with water and dried over sodium sulfate, then the ethyl acetate was evaporated off to give ferulic acid (IIIb) (19 mg) as colorless needles, mp 174—176 °C (methanol–benzene) (Synthesized mp 174 °C).

Acetylation of VIII——VIII (7 mg) was acetylated in the usual manner with pyridine and acetic anhydride.

Recrystallization from methanol gave VIIIa (4 mg), colorless needles, mp 143—144 °C. UV λ_{max}^{MeOH} (log ε): 320 (sh 4.41), 307 (4.54), 286 (sh 4.32), 277 (sh 4.24), 242 (sh 3.82). IR ν_{max}^{KBr} cm^{-1}: 1755, 1615, 1500, 1285, 1195, 1108, 1035, 880, 800. MS *m/z*: 340 (M^+, 19), 298 ($M^+ - CH_2-C=O$, 25), 256 ($M^+ - 2\times CH_2-C=O$, 100), 241 ($M^+ - 2\times CH_2-C=O-CH_3$, 24). ^{1}H-NMR (acetone-d_6) δ: 2.28, 2.41 (each 3H, s, $OCOCH_3$), 3.87 (3H, s, OCH_3), 6.84 (1H, d, J= 2.5 Hz, H-3′), 6.98 (1H, dd, J=9, 2.5 Hz, H-5′), 6.99 (1H, dd, J=8, 2 Hz, H-5), 7.09 (1H, d, J=1 Hz, H-3), 7.33 (1H, m, H-7), 7.58 (1H, d, J=8 Hz, H-4), 7.90 (1H, d, J=9 Hz, H-6′). NOEs were observed at H-3′ (9%) and H-5′ (15%) on irradiation at the methoxyl signal.

Acetylation of X——Crude X (14 mg) was acetylated in the usual manner with pyridine and acetic anhydride. Recrystallization from methanol gave Xa (10 mg), colorless needles, mp 164—168 °C. UV λ_{max}^{MeOH} (log ε): 329 (4.46), 315 (4.54), 302 (sh 4.35), 289 (sh 4.19), 280 (4.16). IR ν_{max}^{KBr} cm^{-1}: 1760, 1750, 1615, 1485, 1370, 1325, 1280, 1208, 1168, 1150, 1105, 805. MS *m/z*: 370.1085 (Calcd for $C_{20}H_{18}O_7$: 370.1055), 328.0942 (Calcd for $C_{18}H_{16}O_6$: 328.0946), 286.0825 (Calcd for $C_{16}H_{14}O_5$: 286.0842), 271.0590 (Calcd for $C_{15}H_{11}O_5$: 271.0607). ^{1}H-NMR (acetone-d_6) δ: 2.34, 2.40 (each 3H, s, $OCOCH_3$), 3.84 (3H, s, OCH_3 at C-4′), 3.88 (3H, s, OCH_3 at C-6), 6.69 (1H, d, J=2.5 Hz, H-3′), 6.78 (1H, d, J=1 Hz, H-3), 6.87 (1H, dd, J=9, 2.5 Hz, H-5′), 7.09 (1H, br s, H-7), 7.19 (1H, s, H-4), 7.81 (1H, d, J=9 Hz, H-6′). NOEs were observed at H-3′ (13%) and H-5′ (19%) on irradiation at the methoxy signal (3.84 ppm) and at H-7 (20%) on irradiation at the methoxy signal (3.88 ppm).

Acknowledgement The authors are indebted to Prof. N. Morita and Dr. M. Arisawa, Toyama Medical and Pharmaceutical University for providing authentic isoflavonoids, to Dr. M. Uchida for measurements of mass spectra and to Mrs. H. Kitamura for the elemental analyses.

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11) Product of Wako Pure Chemical Industries, Ltd.
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