Studies on the Constituents of Indonesian *Picrasma javanica*. III. Structures of New Quassinoids, Javanicins A, C and D

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Three new quassinoids, javanicins A, C and D, were isolated from the bark of *Picrasma javanica* (Simaroubaceae). The structures were established on the basis of spectroscopic data and chemical evidence.

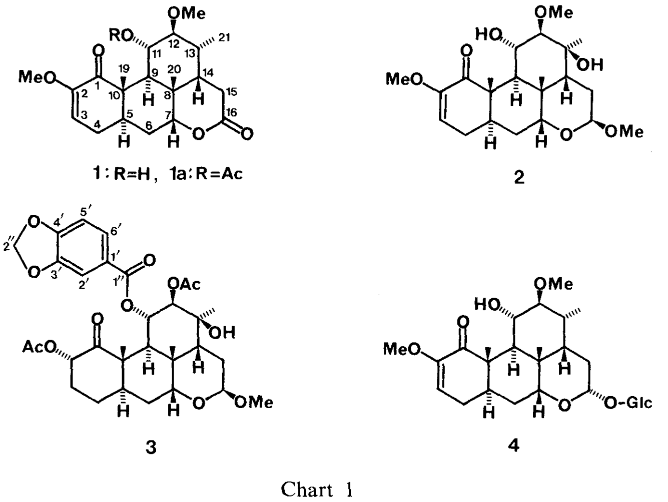
Keywords javanicin A; javanicin C; javanicin D; quassinoid; *Picrasma javanica*; Simaroubaceae

Quassinoids, bitter principles of Simaroubaceous plants, have been extensively investigated from the structural viewpoint, because of their useful biological activity.[1] Isolation and structure elucidation of a new quassinoid glucoside, named javanicinoside A, from the bark of Indonesian *Picrasma javanica* Bl. was reported previously.[2] The absolute stereochemistry of the quassinoid was confirmed by single crystal X-ray structure analysis.[2] Further investigation on the bitter principles of this plant led to the isolation of three new quassinoids from the bark. The present paper deals with the isolation and structure elucidation of these compounds on the basis of spectral data and chemical evidence.

The bark of *P. javanica* collected in Indonesia was extracted with methanol. The chloroform-soluble fraction from the methanol extract was chromatographed on a silica gel column yielding a mixture of quassinoid fractions. Each fraction was further purified by high-performance liquid chromatography (HPLC) on octadecyl silica (ODS) eluted with methanol–water. The isolation procedures afforded three new quassinoids, named javanicins A (**1**), C (**2**) and D (**3**).

Javanicin A (**1**), mp 275 °C, obtained as colorless prisms from acetone, showed $[\alpha]_D^{24}$ +76.1°, and its molecular formula was determined to be $C_{21}H_{30}O_6$ by high-resolution mass spectroscopy (HRMS). Its infrared (IR) and ultraviolet (UV) spectra indicated the presence of hydroxyl (ν_{max} 3540 cm^{-1}), δ-lactone (ν_{max} 1730 cm^{-1}) and α,β-unsaturated carbonyl (ν_{max} 1680, 1640 cm^{-1} and λ_{max} 271 nm) groups. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed signals due to one secondary methyl at δ 0.99 (d, J=8 Hz, 13-Me), two tertiary methyls at δ 1.07 (s, 8-Me) and 1.35 (s, 10-Me), two methoxyls at δ 3.45 (s, 2-OMe) and 3.71 (s, 12-OMe), and an olefinic proton at δ 5.49 (1H, dd, J=6 and 2 Hz, H-3). Acetylation of **1** gave a monoacetate (**1a**) indicating the presence of one hydroxyl group in **1**. From a comparison of the ^{1}H- and carbon-13 (^{13}C)-NMR chemical shift values and J_{HH} coupling constants (J/Hz) with those for reported javanicinoside A (**4**),[2] these data suggested that the acetal function at C-16 in **4** was replaced by δ-lactone carbonyl in **1**. The relative stereochemistry of **1** was determined by nuclear Overhauser effect (NOE) measurements (Fig. 1) as follows. Irradiation of 8-Me protons at δ 1.07 induced NOEs at H-7 (10%), H-11 (12%), H-13 (8%), H-14 (4%), 10-Me (8%) and 12-OMe (6%). Irradiation of the 13-Me protons at δ 0.99 induced 6% NOE at H-12. Irradiation of the H-7 proton at δ 4.10 induced 7% NOE at 8-Me. From these observations, all the angular chiral centers (C-5, C-7, C-8, C-9, C-10 and C-14) together with C-13 were compatible with those of javanicinoside A (**4**) and the usual picrasane skeleton.[1] Thus, the orientations of 11-OH and 12-OMe were expected to be α and β, respectively. On the basis of the above data, the structure of javanicin A was proposed to be formula **1**.

Javanicin C (**2**), mp 201 °C, obtained as colorless prisms from acetone, showed $[\alpha]_D^{24}$ +157.1°, and its molecular formula was determined to be $C_{22}H_{34}O_7$ by HRMS. Its IR and UV spectra indicated the presence of hydroxyl (ν_{max}



1: R=H, **1a**: R=Ac

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Chart 1

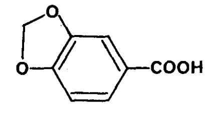

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Chart 2

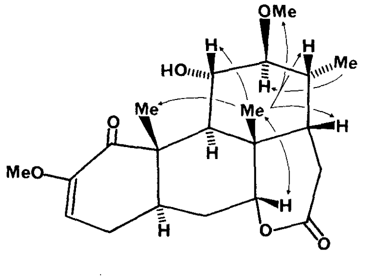

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Fig. 1. NOEs Observed in NOE Difference Spectra of Javanicin A (**1**)

Table I. ^{1}H-NMR Spectral Data[a] for Compounds **1**—**5**

Proton	**1**	**1a**	**4**	**2**	**3**	**5**
H-2					5.49 dd (12, 7)	
H-3	5.49 dd (6, 2)	5.21 dd (5, 2)	5.45 dd (5, 2)	5.56 dd (5, 2)	1.70 m	
H-4	1.86 ddd (18, 6, 4)	1.85 ddd (18, 5, 5)	1.79 ddd (20, 5, 4)	1.99 ddd (18, 5, 4)	1.34 m	
	2.16 ddd (18, 11, 2)	2.05 ddd (18, 11, 2)	2.09 ddd (20, 11, 2)	2.20 ddd (18, 10, 2)	1.75 m	
H-5	2.28 m	2.23 m	2.53 m	2.53 m	2.19 m	
H-6	1.55 ddd (14, 3, 3)	1.54 ddd (14, 3, 3)	1.48 ddd (14, 4, 3)	1.38 ddd (14, 5, 3)	1.44 ddd (14, 3, 3)	
	1.96 ddd (14, 11, 3)	1.96 ddd (14, 11, 3)	1.85 ddd (14, 10, 2)	2.03 ddd (14, 11, 3)	1.96 ddd (14, 11, 3)	
H-7	4.10 t (3)	4.11 t (3)	3.10 t (3)	3.96 t (3)	3.70 t (3)	
H-9	2.43 d (11)	2.85 d (11)	2.75 d (11)	2.81 d (10)	3.62 d (12)	
H-11	3.96 ddd (11, 9, 8)	5.55 dd (11, 10)	3.88 dd (11, 8)	4.37 dd (10, 9)	6.48 dd (12, 10)	
H-12	3.08 dd (10, 8)	3.36 dd (10, 9)	2.86 t (8)	3.16 d (9)	5.67 d (10)	
H-13	2.08 m	2.18 m	2.05 m			
H-14	1.66 ddd (12, 8, 5)	1.69 m	1.17 ddd (13, 4, 4)	2.16 m	2.28 m	
H-15	2.67 dd (20, 8)	2.66 dd (20, 8)	1.56 ddd (14, 13, 9)	1.74 (2H, m)	1.69 m	
	2.76 dd (20, 12)	2.77 dd (20, 12)	1.70 ddd (14, 4, 2)		1.79 m	
H-16			5.15 dd (9, 2)	4.82 d (2)	4.76 d (2)	
8-Me	1.07 s	1.10 s	0.94 s	1.55 s	1.72 s	
10-Me	1.35 s	1.34 s	1.34 s	1.45 s	1.50 s	
13-Me	0.99 d (8)	0.93 d (7)	0.88 d (8)	1.23 s	1.29 s	
11-OH	4.60 d (9)					
2-OMe	3.45 s	3.47 s	3.48 s	3.48 s		
12-OMe	3.71 s	3.39 s	3.68 s	3.72 s		
16-OMe			5.53 d (8, glc-1-H)	3.33 s	3.31 s	
2-OAc			4.05 dd (9, 8, glc-2-H)		2.00 s	
11-OAc		2.07 s	4.20 m (glc-3-H)			
12-OAc			4.20 m (glc-4-H)		1.61 s	
H-2′			3.97 m (glc-5-H)		7.85 d (2)	7.88 d (2)
H-5′			4.43 dd (12, 2, glc-6-H)		6.93 d (8)	6.98 d (8)
H-6′			4.55 dd (12, 2, glc-6-H)		7.99 dd (8, 2)	8.04 dd (8, 2)
H-2″					5.92 d (1)	
					6.01 d (1)	6.05 s

[a] The spectra were measured in pyridine-d_5. The signal assignments were based on spectral analyses of selective ^{1}H decoupling experiments and ^{1}H–^{1}H shift correlation spectra.

3440 cm^{-1}) and α,β-unsaturated carbonyl (ν_{max} 1670, 1635 cm^{-1} and λ_{max} 270 nm) groups. The ^{1}H-NMR of **2** showed the signals due to three tertiary methyls at δ 1.23 (s, 13-Me), 1.45 (s, 10-Me) and 1.55 (s, 8-Me), three methoxyls at δ 3.33 (s, 16-OMe), 3.48 (s, 2-OMe) and 3.72 (s, 12-OMe), and an olefinic proton at δ 5.56 (1H, dd, J=5 and 2 Hz, H-3). The spectral features were very similar to those of the aglycone moiety of javanicinoside A (**4**) except for the absence of the corresponding proton signal due to H-13. Thus, the signal observed at δ 2.05 (1H, m, H-13) in **4** was absent, and the doublet signal of the methyl protons of 13-Me (J=8 Hz) in **4** was transformed into a singlet signal in **2** owing to replacement of the H-13 proton with a hydroxyl group. The relative stereochemistry of **2** was determined by NOE measurements (Fig. 2) as follows. Irradiation of the 8-Me protons at δ 1.55 induced NOEs at H-7 (8%), H-11 (17%), H-14 (19%) and 12-OMe (5%). Irradiation of the 13-Me protons at δ 1.23 induced 10% NOE at H-12. Irradiation of the H-7 proton at δ 3.96 induced NOEs at 8-Me (7%), 10-Me (6%) and 16-OMe (2%). Irradiation of the 16-OMe protons at δ 3.33 induced 4% NOE at H-7. From these observations, all the angular chiral centers (C-5, C-7, C-8, C-9, C-10 and C-14) together with C-13 were concluded to be compatible with those of javanicin A (**1**), javanicinoside A (**4**) and the usual picrasane skeleton. Thus, the orientations of 11-OH, 12-OMe and 16-OMe were determined to be α, β and β, respectively. On the basis of the above data, the structure of javanicin C was proposed to be formula **2**.

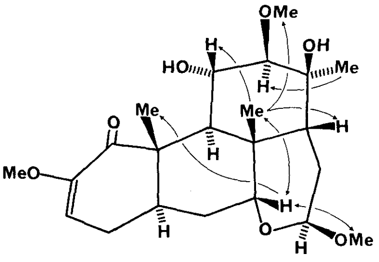

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Fig. 2. NOEs Observed in NOE Difference Spectra of Javanicin C (**2**)

Javanicin D (**3**), mp 124 °C, obtained as colorless needles from acetone, showed $[\alpha]_D^{23}$ −5.3°, and its molecular formula was determined to be $C_{32}H_{40}O_{12}$ by HRMS. The IR spectrum of **3** indicated the presence of hydroxyl (ν_{max} 3450 cm^{-1}), carbonyl (ν_{max} 1745 and 1730 cm^{-1}), phenyl (ν_{max} 1630 and 1480 cm^{-1}) and ester (ν_{max} 1280 and 1260 cm^{-1}) groups. Its UV spectrum (λ_{max} 220, 262 and 296 nm) was similar to that of 3,4-methylenedioxybenzoic acid (**5**)[3] which indicated that javanicin D (**3**) has an aromatic ring in the molecule. The ^{1}H-NMR spectrum of **3** showed three tertiary methyl signals at δ 1.29 (s, 13-Me), 1.50 (s, 10-Me) and 1.72 (s, 8-Me), two acetyl signals at δ 1.61 (s, 12-OAc) and 2.00 (s, 2-OAc), nonequivalent methylene signals at δ 5.92 and 6.01 (each 1H, d, J=1 Hz), and phenyl ring signals at [δ 6.93 (1H, d, J=8 Hz), 7.85 (1H, d, J=2 Hz) and 7.99 (1H, dd, J=8 and 2 Hz)]. The results of ^{1}H–^{1}H shift correlation spectroscopy (COSY) and selective ^{1}H spin

Table II. ^{13}C-NMR Spectral Data[a] for Compounds **1**—**5**

Carbon	**1**	**4**	**2**	**3**	**5**
C-1	204.75	205.86	206.37	208.75	
C-2	149.72	149.65	149.61	73.05	
C-3	112.47	112.38	112.24	34.17	
C-4	28.08	28.28	28.49	25.52	
C-5	36.72	37.78	37.52	43.35	
C-6	29.61	29.71	29.42	29.28	
C-7	82.42	78.26	69.98	69.85	
C-8	37.96	38.15	38.98	38.57	
C-9	37.96	38.59	38.91	37.73	
C-10	48.16	48.64	48.73	52.00	
C-11	74.05	74.36	71.94	71.03	
C-12	88.78	89.69	90.62	79.24	
C-13	34.95	34.95	76.62	75.82	
C-14	44.85	47.75	47.18	48.94	
C-15	28.64	27.74	29.31	29.17	
C-16	169.99	99.64	98.22	98.11	
C-19	11.27	11.30	11.45	12.55	
C-20	21.38	21.50	26.74	25.41	
C-21	14.68	15.22	24.37	25.99	
2-OMe	55.08	54.96	55.04		
12-OMe	61.28	60.86	62.36		
16-OMe			54.22	54.31	
2-OCO$\underline{\text{Me}}$		100.56 (glc-C-1)		20.37	
12-OCO$\underline{\text{Me}}$		75.02 (glc-C-2)		20.37	
2-O$\underline{\text{C}}$OMe		78.29 (glc-C-3)		169.24	
12-O$\underline{\text{C}}$OMe		71.65 (glc-C-4)		170.32	
C-1′		78.64 (glc-C-5)		125.06	126.67
C-2′		62.87 (glc-C-6)		110.86	110.13
C-3′				147.99	148.27
C-4′				151.98	151.69
C-5′				107.96	108.27
C-6′				126.63	125.73
C-1″				165.99	168.99
C-2″				102.24	102.34

[a] The spectra were measured in pyridine-d_5. The signal assignments were based on spectral analyses of ^{1}H–^{13}C and long-range ^{1}H–^{13}C shift correlation spectra.

decoupling NMR experiments on **3** revealed the presence of the partial structures indicated by the bold lines in the formula (Fig. 3). The presence of the 3,4-methylenedioxybenzoyloxyl group was confirmed by spectral comparison with an authentic compound (**5**). Next, a long-range ^{1}H–^{13}C COSY experiment on **3** clarified the sequence of the carbon atoms. As shown in Fig. 3, the tertiary methyl protons at δ 1.50 (10-Me) were correlated with the ketone at δ 208.75 (C-1) and the methine carbon at δ 43.35 (C-5) and quaternary carbon at δ 52.00 (C-10), the tertiary methyl protons at δ 1.72 (8-Me) were correlated with the methine carbons at δ 69.85 (C-7), 37.73 (C-9), 48.94 (C-14) and the quaternary carbon at δ 38.57 (C-8), and the tertiary methyl protons at δ 1.29 (13-Me) were correlated with the methine carbons at δ 79.24 (C-12), 48.94 (C-14) and quaternary carbon at δ 75.82 (C-13). Some of the significant ^{1}H–^{13}C long-range correlations are indicated by arrows in the formula in Fig. 3. Thus, the locations of the 3,4-methylenedioxybenzoyloxyl group and two acetoxyl groups were determined to be at C-11, C-2 and C-12, respectively. The relative stereochemistry of **3** was determined by NOE measurements (Fig. 4) as follows. Irradiation of the 8-Me protons at δ 1.72 induced NOEs at H-7 (7%), H-11 (15%), H-14 (10%), 10-Me (8%) and 12-OAc (7%). Irradiation of the H-7 proton at δ 3.70 induced NOEs at H-14 (9%), 8-Me (5%) and 16-OMe (1%). Irradiation of the 10-Me protons at δ 1.50 induced 24% NOE at H-2. Irradiation of the 2-OAc protons at δ 2.00 induced 7% NOE at H-5. Irradiation of the 12-OAc protons at δ 1.61 induced NOEs at H-11 (14%), H-14 (20%) and 8-Me (12%). Irradiation of the 13-Me protons at δ 1.29 induced 5% NOE at H-12. Irradiation of the 16-OMe protons at δ 3.31 induced 4% NOE at the H-7. From these observations, all the angular chiral centers (C-5, C-7, C-8, C-9, C-10 and C-14) together with C-13 were concluded to be compatible with those of javanicin C (**2**) and javanicinoside A (**4**). Thus the proton at C-2 (δ 5.49) was assigned as β-axial from the coupling constants (J = 12 and 7 Hz), which was confirmed by the presence of an NOE with C-10 β-axial methyl proton signals. From the coupling constants between H-11 and H-9, and H-11 and H-12 (12 and 10 Hz, respectively), the H-11 and H-12 protons were deduced to be β-axial and α-axial, respectively. On the basis of the above data, the structure of javanicin D was proposed to be formula **3**.

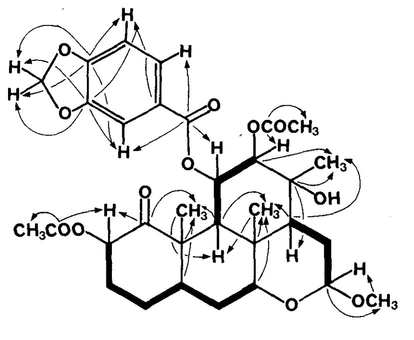

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Fig. 3. Partial Structures and Two- and Three-Bond (C, H) Correlation Pattern of Javanicin D (**3**)

Bold line: partial structure. ↷: long-range ^{1}H–^{13}C coupling.

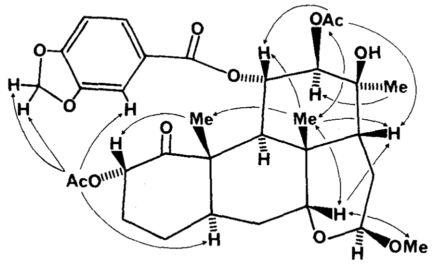

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Fig. 4. NOEs Observed in NOE Difference Spectra of Javanicin D (**3**)

Experimental

The melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. The UV and IR spectra were recorded on Hitachi 340 and Hitachi 260-60 spectrophotometers, respectively. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a JEOL JNM GX-400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer. Chemical shifts are given on the δ-scale (ppm downfield from tetramethylsilane as an internal standard) and coupling constants (J) in hertz (Hz). Electron-ionization (EI)MS and HRMS were run on JEOL JMS D-300 and DX-303 mass spectrometers, respectively. Optical rotations were determined on a JASCO DIP-4 digital polarimeter. Column chromatography was carried out on silica gel (BW-820MH, Fuji Davison). HPLC was carried out on an ODS column (Capcell pak ODS, Shiseido, 10 mm i.d. × 250 mm).

Extraction and Isolation Dried bark (1.8 kg) of *P. javanica* collected at Kebun Raya Bogor, Indonesia, in July, 1986, was extracted with MeOH (16 l). The extract was concentrated under reduced pressure to give a residue (263 g), to which an equal volume of water was added. The aqueous solution was extracted with $CHCl_3$ (4 l) and then with *n*-BuOH (4 l). The $CHCl_3$-soluble fraction (60 g) was applied to a column of silica gel (900 g). Elution was performed with 1, 5, 10, 20 and 50% MeOH in $CHCl_3$ and with MeOH. The 5% MeOH in $CHCl_3$ fraction was subjected to preparative HPLC using Capcell pak ODS with a mixed solvent, H_2O–MeOH (3 : 2, v/v), to afford javanicin A (**1**, 75 mg), javanicin C (**2**, 7 mg) and javanicin D (**3**, 17 mg).

Javanicin A (1) Colorless prisms (acetone), mp 275 °C, $[\alpha]_D^{24}$ $+76.1°$ (c=0.8, MeOH). UV λ_{max}^{MeOH} nm (log ε): 271 (4.31). IR ν_{max}^{KBr} cm^{-1}: 3540, 1730, 1680, 1640, 1240, 1105, 1040. ^{1}H-NMR and ^{13}C-NMR data are given in Tables I and II, respectively. MS *m/z* (%): 378 (M$^+$, 14), 346 (29), 330 (30), 315 (55), 312 (59), 287 (15), 271 (8), 239 (13), 203 (44), 191 (11), 98 (100). HRMS: Found, *m/z* 378.2040. Calcd for $C_{21}H_{30}O_6$, *m/z* 378.2042.

Acetylation of Javanicin A (1) Javanicin A (**1**, 10 mg) was acetylated with acetic anhydride (2 ml) and pyridine (2 ml) at 140 °C for 18 h. After MeOH (5 ml) was added, the reaction mixture was evaporated *in vacuo* to give a residue, which was separated by column chromatography (silica gel, 5 g). Elution with $CHCl_3$–MeOH (15 : 1) afforded monoacetyljavanicin A (**1a**, 10 mg). **1a**: mp 185 °C, $[\alpha]_D^{24}$ $+65.4°$ (c=0.8, MeOH). IR ν_{max}^{KBr} cm^{-1}: 1730, 1700, 1640, 1250, 1112, 1045. ^{1}H-NMR data are given in Table I. MS *m/z* (%): 420 (M$^+$, 4), 388 (9), 360 (50), 346 (100), 331 (10), 317 (23), 287 (11), 285 (15), 269 (14), 203 (41), 171 (10).

Javanicin C (2) Colorless prisms (acetone), mp 201 °C, $[\alpha]_D^{24}$ $+157.1°$ (c=0.7, MeOH). UV λ_{max}^{MeOH} nm (log ε): 270 (3.44). IR ν_{max}^{KBr} cm^{-1}: 3440, 1670, 1635, 1240, 1105, 1040. ^{1}H-NMR and ^{13}C-NMR data given in Tables I and II, respectively. MS *m/z* (%): 410 (M$^+$, 6), 392 (18), 375 (100), 360 (17), 345 (12), 283 (10), 254 (9), 154 (48), 43 (52). HRMS: Found, *m/z* 410.2322. Calcd for $C_{22}H_{34}O_7$, *m/z* 410.2305.

Javanicin D (3) Colorless needles (acetone), mp 124 °C, $[\alpha]_D^{23}$ $-5.3°$ (c=1.1, MeOH). UV λ_{max}^{MeOH} nm (log ε): 220 (3.94), 262 (3.52), 296 (3.45). IR ν_{max}^{KBr} cm^{-1}: 3450, 1745, 1730, 1630, 1480, 1280, 1260, 1050. ^{1}H-NMR and ^{13}C-NMR data are given in Tables I and II, respectively. MS *m/z* (%): 616 (M$^+$, 25), 575 (6), 425 (21), 365 (7), 287 (10), 222 (7), 166 (10), 149 (100), 43 (56). HRMS: Found, *m/z* 616.2505. Calcd for $C_{32}H_{40}O_{12}$, *m/z* 616.2520.

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References and Notes

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2) Part II: T. Ohmoto, K. Koike, K. Mitsunaga, H. Fukuda, K. Kagei, T. Kawai and T. Sato, *Chem. Pharm. Bull.*, **37**, 993 (1989).
3) 3,4-Methylenedioxybenzoic acid (piperonylic acid, **5**): UV λ_{max}^{MeOH} nm (log ε): 216 (4.07), 258 (3.58) and 296 (3.52).