Tannins and Related Compounds. CVII.[1] Structure Elucidation of Three New Monomeric and Dimeric Ellagitannins, Flosin B and Reginins C and D, Isolated from *Lagerstroemia flos-reginae* RETZ.

Ya-Ming XU,[a] Takashi TANAKA,[b] Gen-ichiro NONAKA*,[b] and Itsuo NISHIOKA[b]

Shanghai Institute of Materia Medica, Academica Sinica,[a] 319 Yueyan Road, Shanghai, Peoples' Repulic of China and Faculty of Pharmaceutical Sciences, Kyushu University 62,[b] 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan. *Received October 3, 1990*

Further chemical work on tannins in the leaves of *Lagerstroemia flos-reginae* RETZ. (Lythraceae) has resulted in the isolation of new monomeric (flosin B) and dimeric ellagitannins (reginins C and D), together with pterocarinin A (1) and 5-desgalloylpterocarinin A (2). On the basis of chemical and spectroscopic evidence, the structure of flosin B was determined to be a *C*-glycosidic ellagitannin (3) possessing a valoneic acid dilactonyl group, while reginins C and D were characterized as dimeric ellagitannins (6 and 10, respectively), in which a pedunculagin moiety is connected to pterocarinin A and casuarinin [the C-1 epimer of stachyurin (5)] moieties, respectively, through a carbon-to-oxygen bond.

Keywords *Lagerstroemia flos-reginae*; Lythraceae; *C*-glycosidic ellagitannin; flosin B; reginin C; reginin D; biomimetic synthesis; L-ascorbic acid; tannin

In the preceding paper,[1] we reported the isolation and characterization of eighteen hydrolyzable tannins and related compounds, including four new hydrolyzable tannins [reginins A (**7**) and B (**8**), flosin A and lagerstroemin (**4**)], from the leaves of *Lagerstroemia flos-reginae* RETZ. (Lythraceae). Further chemical examination of tannins in this plant has resulted in the isolation of three new ellagitannins [flosin B (**3**) and reginins C (**6**) and D (**10**)], together with two known compounds [pterocarinin A (**1**) and 5-desgalloylpterocarinin A (**2**)]. This paper deals with the isolation and structure elucidation of these compounds.

A combination of Sephadex LH-20, MCI-gel CHP 20P, TSK gel Toyopearl HW 40F and TSK gel Phenyl Toyopearl 650M chromatographies of fraction 2, which was previously obtained from the aqueous acetone extract of the dried leaves, afforded flosin B (**3**), reginins C (**6**) and D (**10**), pterocarinin A (**1**),[2] and 5-desgalloylpterocarinin A (**2**).[2]

Flosin B (**3**), an off-white amorphous powder, $[\alpha]_D$ +65° (MeOH), gave positive ferric chloride and nitrous acid tests,[3] characteristic of ellagitannins. The negative fast atom bombardment mass spectrum (FAB-MS) gave the same $[M-H]^-$ peak at *m/z* 1235 as that of previously reported lagerstroemin (**4**). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed six aromatic one-proton singlets, of which the chemical shifts of three signals (δ 7.50, 7.20 and 7.19) were similar to those (δ 7.64, 7.20 and 7.14) found in **4**, a finding consistent with the presence of a valoneic acid dilactonyl group. The aliphatic signal pattern was typical of a *C*-glycosidic ellagitannin, showing no anomeric proton signal, and the chemical shifts and coupling constants agreed well with those of stachyurin (**5**).[4] Among these signals, the small coupling constant of the *C*-glycosidic H-1 signal [δ 5.02 (d, J=1.5 Hz)] clearly indicated that **3** possesses the same configuration at the C-1 position as that of **5**. Furthermore, the chemical shifts of the aliphatic signals in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum were almost in line with those of **5**, while among seven carboxyl carbon resonances, two upfield signals (δ 160.8 and 161.2) were characteristic of the δ-lactone carbons in the valoneoyl group. Thus, these findings suggested that flosin B has the same configuration in the *C*-glycoside moiety as that of **5**, and is the C-1 epimer of lagerstroemin (**4**).

Epimerization[5] at the *C*-glycosidic C-1 position was carried out by heating **4** in aqueous solution to give **3** in 15% yield. Accordingly, the structure of flosin B was unequivocally established as **3**.

Reginin C (**6**) was isolated as an off-white amorphous powder, $[\alpha]_D$ +53.8° (MeOH). The ^{1}H-NMR spectrum was extremely complicated. However, the chemical shifts of aromatic signals, although the signals were partly split, were found to be almost the same as those of the previously reported dimeric ellagitannin, reginin B (**8**). The aliphatic signal pattern between δ 4.0—6.7 was also closely correlated with that of **8**. The observation of additional complex signals at δ 3.5—4.0 indicated the existence of an extra polyalcohol

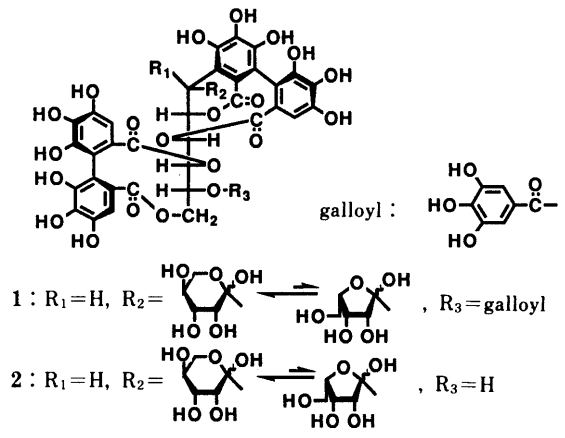


Chart 1

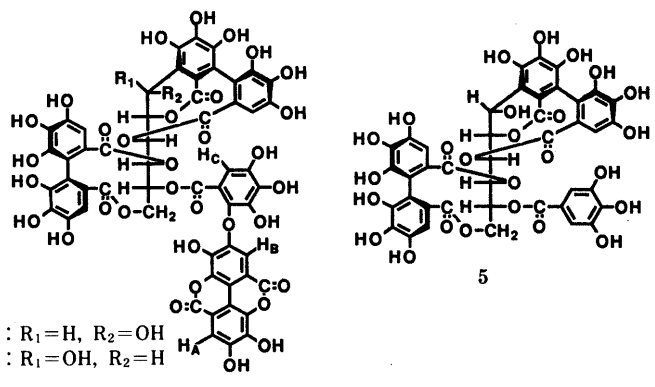


Chart 2

moiety carrying no acyl group. Although the aromatic signal pattern in the ^{13}C-NMR spectrum was complicated, the aliphatic signals arising from the major conformers could be assigned as shown in Table I by comparison with those of pterocarinin A(**1**) and pedunculagin (**9**). Among these, the signal at δ 102.1 was assignable to a ketal carbon, and the signal at δ 46.3 to the C-1 atom of an open-chain form of the glucose core to which the extra polyalcohol moiety is connected through a carbon-to-carbon bound. Furthermore, the observation of a pair of doublets at δ 95.0 and 91.5 was indicative of the presence of β- and α-forms of the glucopyranose cores. From these observations, reginin C was considered to be a dimeric ellagitannin in which pterocarinin A (**1**) and pedunculagin (**9**) moieties are linked through a carbon-to-oxygen bond. This consideration was also supported by the observation of the intense $[M-H]^-$ peak at m/z 1849 in the negative FAB-MS.

In order to confirm the structure, the synthesis of reginin C was carried out by condensation of L-ascorbic acid and reginin B (**7**),[2] giving reginin C in 20% yield. Therefore, the structure of reginin C was determined unambiguously to be as represented by the formula **6**.

Reginin D (**10**) was isolated as an off-white amorphous powder, $[\alpha]_D$ +82° (MeOH). The negative FAB-MS showed the same $[M-H]^-$ peak at m/z 1717 as those of reginins A (**7**) and B (**8**). The ^{1}H-NMR spectrum was duplicated owing to the presence of an α- and β-anomeric mixture. The aromatic signals all appearing as singlets corresponded to eight protons in total. The ^{13}C-NMR spectrum (Table II) of **10** showed aliphatic signal patterns similar to those of reginin A (**7**), and the signals at δ 94.8 and 91.3 were attributable to the β- and α-anomeric carbons in the glucopyranose moiety.

To avoid tautomerism, reginin D was treated with *p*-anisidine in ethanolic acetic acid, followed by sodium cyanoborohydride reduction.[1] The ^{1}H-NMR spectrum of the aminoalditol derivative (**14**) thus obtained was simplified, and clearly showed signal patterns closely correlated with those of the reginin A aminoalditol (**15**), although a moderate upfield shift (δ 6.25, ref. δ 6.58 in **15**) of one (H-B in the valoneoyl group) of the aromatic signals was observed in **14**.[1]

Partial hydrolysis of **10** in hot water afforded lagerstroemin (**4**) and 4,6-(*S*)-hexahydroxydiphenoyl(HHDP)-D-glucose (**13**). This result indicated that the valoneoyl group is located at the glucopyranose C-2 and C-3 positions in **10**. Furthermore, methylation of **10** with dimethyl sulfate and potassium carbonate in dry acetone, followed by alkaline methanolysis with 2% sodium methoxide, gave dimethyl (*S*)-hexamethoxydiphenoate (**11**) and trimethyl octa-*O*-methyl-(*S*)-valoneate (**12**). Thus, the atropisomerism of the valoneoyl ester group in **10** was established to be in the *S*-series. From these chemical and spectroscopic findings, the structure of reginin D was determined to be 10. The orientation of the valoneoyl group still remains to

Table I. ^{13}C-NMR Data for Reginin C (**6**), Pterocarinin A (**1**), Reginin B (**8**) and Pedunculagin (**9**)

	6[a,c]	1[a,c]	8[a]	9[b]
1″	102.1	102.0		
2″	72.2	72.2		
3″	72.2	72.2		
4″	66.9	66.9		
5″	63.2	63.2		
1′	46.3	46.4	64.7	
2′	75.5	75.3	81.6	
3′	74.3	74.2	72.3	
4′	73.9	73.5	73.7	
5′	70.7	71.1	71.1	
6′	64.4	64.5	64.4	
1 α	91.5		91.4	91.8
β	95.0		94.9	95.4
2 α	75.5		75.5	75.6
β	78.2		78.2	78.3
3 α	75.7		75.7	75.8
β	77.2		77.2	77.6
4 α	70.4		70.4	69.6
β	70.8		70.9	69.9
5 α	67.5		67.5	67.4
β	72.3		72.2	72.5
6 α	64.0		64.4	63.6
β	64.0		64.4	63.6

Acetone-d_6+D_2O, ppm, TMS. *a*) Spectra measured at 67.8 MHz. *b*) Spectrum measured at 25.05 MHz. *c*) Only the signals of major conformers are given.

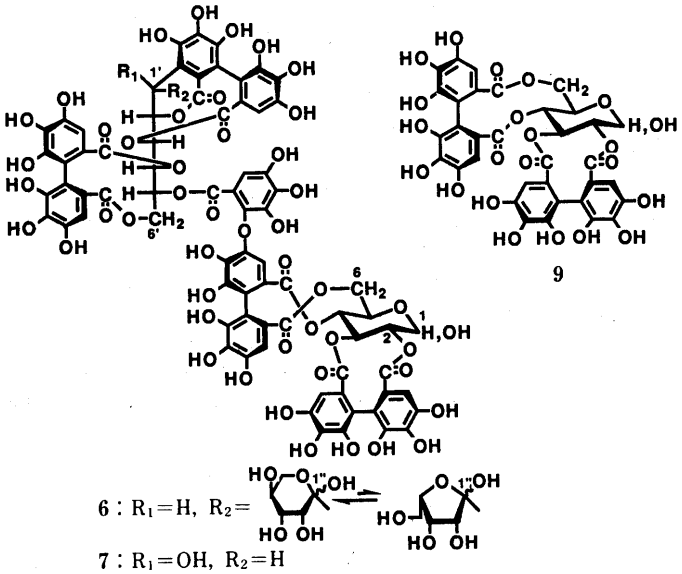
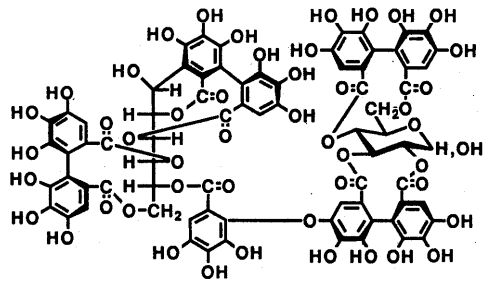


Chart 3

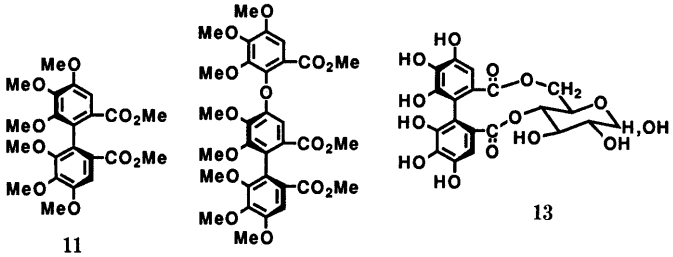
Table II. ^{13}C-NMR Data for Reginins D (**10**) and A (**7**)

		C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
10	β	94.8	78.5	77.0	69.9	72.2	63.8	67.0	77.4	69.9	74.1	71.3	64.3
	α	91.3	75.6	75.6	69.8	67.2	63.8						
7	β	95.3	78.8	77.6	70.9	72.7	64.4	67.8	77.5	70.3	74.6	71.5	65.0
	α	91.8	76.0	76.2	70.3	67.5	64.4						

67.8 MHz, acetone-d_6+D_2O, ppm, TMS.

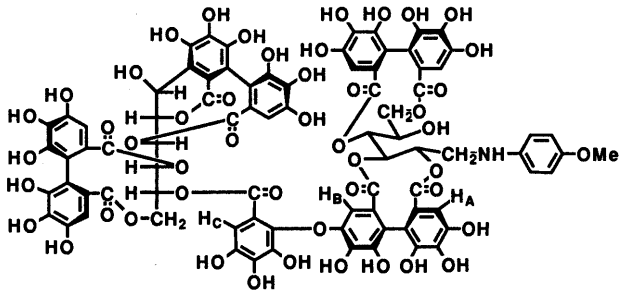


10

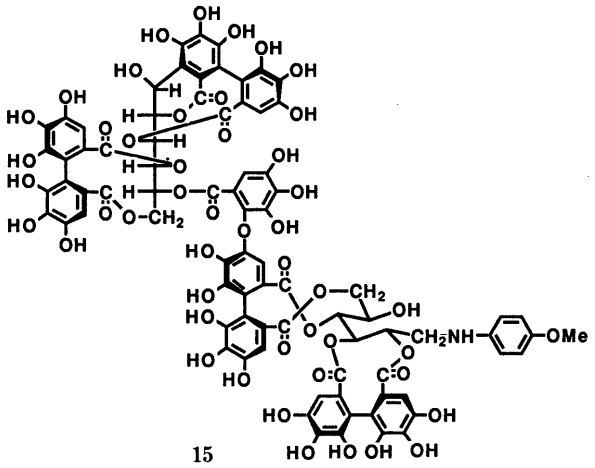


11 12 13

Chart 4



14



15

be proved.

Reginin C (**6**) is the first example of a dimeric ellagitannin containing a C_5-polyalcohol moiety at the C-1 position of the open-chain form of glucose.

Experimental

Details of the instruments and chromatographic conditions used throughout this work are the same as described in the previous paper.[1]

Isolation of Tannins Extraction and fractionation procedures were described in the preceding paper.[1] On chromatography over Sephadex LH-20, MCI-gel CHP 20P, TSK gel Toyopearl HW-40F and TSK gel Phenyl Toyopearl 650M with H_2O–MeOH, fraction 2-a yielded 5-desgalloylpterocarinin A (**2**) (50 mg) and reginin C (**6**) (70 mg), fraction 2-b afforded pterocarinin A (**1**) (70 mg) and fraction 2-c gave flosin B (**8**) (50 mg) and reginin D (**10**) (100 mg).

Flosin B (3) An off-white amorphous powder, $[\alpha]_D^{26}$ +65° (c=1.4, MeOH). *Anal.* Calcd for $C_{55}H_{32}O_{34}$: C, 51.72; H, 2.91. Found: C, 51.69; H, 3.09. Negative FAB-MS m/z: 1235 $[M-H]^-$. ^{1}H-NMR (270 MHz, acetone-d_6+D_2O): 7.56 (1H, s, valoneoyl H-A), 7.20, 7.29 (each 1H, s, valoneoyl H-B and H-C), 6.88, 6.51, 6.40 (each 1H, s, arom. H), 5.57 (1H, dd, J=2, 8 Hz, glc. H-4), 5.21 (1H, dd, J=3, 8 Hz, glc. H-5), 5.02 (1H, d, J=1.5 Hz, glc. H-1), 5.01 (1H, t, J=2 Hz, glc. H-3), 4.80 (1H, dd, J=1.5, 2 Hz, glc. H-2), 4.75 (1H, dd, J=3, 13 Hz, glc. H-6), 3.77 (1H, d, J=13 Hz, glc. H-6). ^{13}C-NMR (25.05 MHz, acetone-d_6+D_2O): 169.2 (×2), 168.7, 166.7, 164.4 (–COO–), 161.2, 160.8 (carboxyl C of δ-lactone), 150.1, 149.4 (arom. C), 81.4 (C-2), 72.9 (C-4), 72.0 (C-3), 70.9 (C-5), 64.7 (C-1), 64.4 (C-6).

Epimerization of Lagerstroemin (4) A solution of **4** (50 mg) in water was heated at 80 °C for 20 h. The reaction product was purified on an MCI-gel CHP 20P column with H_2O–MeOH to afford flosin B (**3**) (8 mg) and the starting material (**4**) (20 mg).

Reginin C (6) An off-white amorphous powder, $[\alpha]_D^{30}$ +53.8° (c=0.9, MeOH). *Anal.* Calcd for $C_{80}H_{58}O_{52}\cdot H_2O$: C, 51.39; H, 3.21. Found: C, 51.15; H, 3.24. Negative FAB-MS m/z: 1849 $[M-H]^-$. ^{1}H-NMR (270 MHz, acetone-d_6+D_2O): 7.02, 7.01 (1H in total, each s, arom. H), 6.64, 6.63 (1H in total, each s, arom. H), 6.60 (1H, s, arom. H), 6.59, 6.58 (1H in total, each s, arom. H), 6.49, 6.48 (1H in total, each s, arom. H), 6.30 (1H, s, arom. H), 6.29, 6.24 (1H in total, each s, arom. H), 3.50—5.70 (19H, m, polyalcohol-H). ^{13}C-NMR (67.8 MHz, acetone-d_6+D_2O): 169.5, 169.4, 169.3, 169.2, 168.8, 168.7, 167.1, 164.8, 164.7 (–COO–). For other signals, see Table I.

Condensation of Reginin B (7) with L-Ascorbic Acid, Giving Reginin C (6) A mixture of **7** (60 mg) and L-ascorbic acid (30 mg) in 0.1 M acetic acid was heated at 80 °C for 7 h. The reaction product was separated by MCI-gel CHP 20P chromatography with H_2O–MeOH (4:1) to give the crude product, which was further purified on a Cosmosil 75C_{18}-OPN column with H_2O–MeOH to yield reginin C (**6**) (12 mg).

Reginin D (10) An off-white amorphous powder, $[\alpha]_D^{26}$ +82° (c=1.8, MeOH). *Anal.* Calcd for $C_{75}H_{50}O_{48}\cdot 3H_2O$: C, 50.79; H, 3.27. Found: C, 50.83; H, 3.09. Negative FAB-MS m/z: 1717 $[M-H]^-$. ^{1}H-NMR (100 MHz, acetone-d_6+D_2O): 7.04 (1H, s, valoneoyl H-C), 6.83, 6.80 (1H in total, each s, arom. H), 6.65 (1H, s, arom. H), 6.63, 6.61 (1H, in total, each s, arom. H), 6.57 (1H, s, arom. H), 6.54, 6.53 (1H in total, each s, arom. H), 6.32 (1H, s, arom. H), 6.25, 6.21 (1H in total, each s, arom. H), 3.50—5.70 (14H in total, m, polyalcohol-H). ^{13}C-NMR (25.05 MHz, acetone-d_6+D_2O): 170.2, 169.8, 169.5, 168.8, 168.7, 168.4, 168.0, 165.2, 165.0, 164.5, 164.2 (–COO–). For other signals, see Table II.

Methylation of 10, Followed by Alkaline Methanolysis A mixture of **10** (50 mg), dimethyl sulfate (1 ml) and anhydrous potassium carbonate (1.0 g) in dry acetone (10 ml) was heated under reflux for 5 h. After removal of the inorganic salts by filtration, the filtrate was concentrated to dryness, and the oily residue was chromatographed over silica gel with benzene containing increasing proportions of acetone to afford a methylate mixture (25 mg), which was treated with 2% sodium methoxide in dry methanol at room temperature for 24 h. After neutralization with Amberlite IR 120B (H^+ form) and concentration *in vacuo*, the residue was chromatographed over silica gel with benzene–acetone to give dimethyl (*S*)-hexamethoxydiphenoate (**11**) (5 mg) and trimethyl octa-*O*-methyl-(*S*)-valoneate (**12**) (1 mg).

Preparation of the Aminoalditol Derivative (14) A mixture of **10** (20 mg) and *p*-anisidine (5 mg) in 20% ethanolic acetic acid (1 ml) was kept at room temperature for 2 h with stirring. The reaction mixture was treated with sodium cyanoborohydride (5 mg) at room temperature for 1 h. The product was purified by Sephadex LH-20 chromatography with EtOH–H_2O to furnish the aminoalditol (**14**). An off-white amorphous powder, $[\alpha]_D^{30}$ +79.3° (c=0.6, MeOH). *Anal.* Calcd for $C_{82}H_{57}O_{48}\cdot H_2O$: C, 52.93; H, 3.28; N, 0.75. Found: C, 52.92; H, 3.39; N, 0.79. ^{1}H-NMR (270 MHz, acetone-d_6+D_2O): 7.02 (1H, s, valoneoyl H-C), 6.84, 6.69, 6.60, 6.58, 6.57 (each 1H, s, arom. H), 6.58 (5H, s, anisidine- and HHDP-H), 6.25 (1H, s, valoneoyl H-B), 5.58 (1H, d, J=5 Hz, glc. H-1′), 5.51 (1H, dd, J=1.5, 9 Hz, glc. H-3), 5.35—5.45 (3H, m, glc. H-2′, 4′, and 5′), 5.25 (1H, m, glc. H-2), 5.21 (1H, dd, J=8, 1 Hz, glc. H-4), 4.95 (1H, dd, J=2.5, 13 Hz, glc. H-6′), 4.70 (1H, dd, J=9, 13 Hz, glc. H-6), 4.64 (1H, dd, J=1.5, 5 Hz, glc. H-2′), 4.27 (1H, dd, J=2, 8 Hz, glc. H-5), 3.50—4.10 (overlapped with HOD signals, glc. H-6′, 6 and 1), 3.61 (3H, s, OMe), 3.42 (1H, dd, J=8, 13 Hz, glc. H-1).

Partial Hydrolysis of 10 An aqueous solution (1 ml) of **10** (20 mg) was heated at 80 °C for 20 h. The reaction mixture was directly subjected to MCI-gel CHP 20P chromatography with H_2O–MeOH to give lagerstroemin (**4**) (5 mg) and 4,6-HHDP-D-glucose (**13**) (2 mg).

Acknowledgements The authors would like to thank P. T. Eisai Indonesia for the supply of plant materials, Mr. Y. Tanaka and Miss Y. Soeda for ^1H- and ^{13}C-NMR spectral measurements, Mr. R. Isobe for MS measurements and the staff of the Central Analysis Room of this University for elemental analysis. Y. M. Xu is grateful to Nippon Mektron Co., for the award of a scholarship.

References

1) Part CVI: Y.-M. Xu, T. Sakai, T. Tanaka, G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **39**, 1639 (1991).
2) G. Nonaka, K. Ishimaru, R. Azuma, M. Ishimatsu and I. Nishioka, *Chem. Pharm. Bull.*, **37**, 2071 (1989).
3) E. C. Bate-Smith, *Phytochemistry*, **11**, 1153 (1972).
4) G. Nonaka, T. Sakai, T. Tanaka, K. Mihashi and I. Nishioka, *Chem. Pharm. Bull.*, **38**, 2151 (1990).
5) W. Mayer, W. Babler, A. Riester and H, Korger, *Justus Liebigs Ann. Chem.*, **707**, 117 (1967); T. Tanaka, G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **34**, 656 (1986).