行政院國家科學委員會補助專題研究計畫成果報告

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※ **櫰仁及小葉櫰仁樹皮鞣質之研究** ※
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□國際合作研究計畫國外研究報告書一份

執行單位：台北醫學大學藥學系

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中文摘要

在系統地研究使君子科值物之鞣質化合物過程，從欖仁樹皮分離出一個新化合物 catappanin A,**兩個** phenolcarboxylic acids, **兩個** phenol glucoside gallates, **七個** ellagic tannins, **一個其他之加水分解型鞣質**, **四個** flavan-3-ols **及兩個混合型鞣質。另外，從小葉欖仁樹皮得到一個新化合物** methyl glucoside gallate **及** methyl 3,6-di-*O*-galloyl-β-D-glucoside, **三個** phenolcarboxylic acids, **三個** gallotannins, **六個** ellagitannins **及三個其他之加水分解型鞣質。 藉由化學或光譜學證據解析其化學結構。**

關鍵詞：欖仁、小葉欖仁、君子科、鞣質、Catappanin A、 Methyl 3,6-di-*O*-galloyl-β-D-glucoside.

ABSTRACT

Continuing chemical examination on tannins and related compounds of Combretaceous plants has led to the isolation of one novel complex type tannin, catappanin A, together with two phenolcarboxylic acids, two phenol glucoside gallates, seven ellagic tannins, one other hydrolyzable tannin, four flavan-3-ols and two complex type tannins from the bark of *Terminalia catappa*. In addition, from the bark of *Terminalia parviflora* one new methyl glucoside gallate, methyl 3,6-di-*O*-galloyl- β-D-glucoside, three phenolcarboxylic acids, three gallotannins, six ellagitannins and three other hydrolyzable tannins were isolated. Their structures were elucidated on the basis of chemical and spectroscopic evidence.

Keywords: *Terminalia catappa*; *Terminalia parviflora*, Combretaceae, Tannin, Catappanin A, Methyl 3,6-di-*O*-galloyl-β-D-glucoside.

INTRODUCTION

It has been documented that the tannins contained esters of triphenic acid (flavogallonic acid and valoneaic acid) and tetraphenic acid (gallagic acid and terchebulic acid) from *Anogeissus acuminata*,[1] *Lumnitzera racemosa*,[2] *Quisqualis indica*,[3] *Terminalia chebula*,[4] *Terminalia arjuna*,[5] and *Terminalia arborea*.[6] Almost all the Combretaceous plants so far examined have been found to contain surprisingly large amounts of castalagin, punicalin and punicalagin. In continuing our chemical studies on tannins in the plants of Combretaceae, we examined the bark of *Terminalia catappa* L. and *Terminalia parviflora* Presl.

T. catappa is broadly distributed on tropical and subtropical beaches and grows natively in southern Taiwan and the Orchid Islet. The constituents of the leaves of this plant are chiefly hydrolyzable tannins such as punicalin, punicalagin, terflavins A and B, and tercatein.[7] *T. parviflora* originates in Sri Lanka and India, and has been used as folk medicine for diarrhea in Sri Lanka.

This paper deals with the isolation and characterization of the tannins from the bark of these two plants mentioned above.

RESULTS AND DISCUSSION

The 80% aqueous acetone extracts of the dried bark of *T. catappa* and *T. parviflora* were respectively subjected to a combination of polydextran, high porous polystyrene and reverse-phase column chromatography with various solvent systems as described in the experimental section. These efforts resulted in isolation of nineteen compounds (**1**-**19**) from the bark of *T. catappa*. Compounds **1**-**18** were identified as: gallic acid (**1**),[8] ellagic acid (**2**),[9] 2,3-(*S*)-HHDP-D-glucose (**3**),[10] punicalagin (**4**),[11] corilagin (**5**),[12] tercatain (**6**),[7] casuarinin (**7**),[13] castalagin (**8**),[13] grandinin (**9**),[13,14] castalin (**10**),[15] 3-methoxy-4-hydroxyphenol-1-*O*-β-D-(6'-*O*-galloyl)-glucoside (**11**),[16] 3,5-dimethoxy-4-hydroxy-phenol-1-*O*-β-D-(6'-*O*-galloyl)-glucoside (**12**),[16] (-)-epicatechin-3-*O*-gallate (**13**),[17] (-)-

epigallocatechin-3-*O*-gallate (**14**),[18] procyanidin B-l (**15**),[19] 3'-*O*-galloyl procyanidin B-2 (**16**),[18] acutissimin A (**17**),[13,20] and eugenigrandin A (**18**).[21] From the bark of *T. parviflora*, gallic acid (**1**), ellagic acid (**2**), 2,3-(*S*)-(HHDP)-D-glucose (**3**), punicalagin (**4**), castalagin (**8**), grandinin (**9**), castalin (**10**), flavogallonic acid (**20**),[15] 1,6-di-*O*-galloyl-β-D-glucose (**21**),[22] 3,6-di-*O*-galloyl-D-glucose (**22**).[23] 1,3,6-tri-*O*-β-D-glucose (**23**),[22] 2,3-(*S*)-HHDP-6-*O*-galloyl-D-glucose (**24**),[24] castamollinin (**25**),[1,25] punicalin (**26**),[11] 2-*O*-galloyl punicalin (**27**)[11] and compound **28** were isolated. Structural elucidation of these compounds was based on spectral analysis and chemical correlation.

Compound **19** was obtained as a pale brown amorphous powder. With the ferric chloride reagent it gave a dark blue coloration as typical hydrolyzable tannins show. The observation of negative coloration in the nitrous acid test[26] revealed that **19** has no HHDP (Hexahydroxydiphenoyl) group. In addition, a reddish pink coloration with the anisaldehyde-sulfuric acid reagent suggested the presence of a flavan-3-ol skeleton in the molecule to associate **19** with a complex type tannin.[16] The ^{1}H NMR spectrum of **19** showed the signals due to a flavan-3-ol and polyalcohol moieties whose chemical shifts and coupling pattern were similar to those of acutissmin C (**29**),[27] indicating the presence of a castalin (**10**)[15,28] constitution in both molecules.

The only difference between the ^{1}H NMR spectra of **19** and **29** was the signals arising from the B-ring of flavan-3-ol moiety. Namely, the proton due to the B-ring of **l9** resonances at δ 6.48 (2H, s) as well as in the case of (+)-gallocatechin (**30**), and the appearance of ABX-type signals at δ 6.90 (1H, br s, H-2'), δ 6.86 (1H, d, *J*=8 Hz, H-6') and δ 6.78 (1H, br d, *J*=8 Hz, H-5') in **29** indicated that the flavan-3-ol was of (+)-catechin (**31**) type. This was supported by the negative FAB-MS of **19,** which displayed the $[M-H]^-$ ion peak at *m/z* 919, sixteen mass units larger than that of acutissimin C (**29**: *m/z* 903). Furthermore, the ^{13}C NMR data of **19** for flavan C-ring carbons (δ 82.1, C-2; δ 68.1, C-3; δ 27.6, C-4) implied the 2,3-*trans* confi-guration of flavan-3-ol unit, while the low-field shift of the A-ring C-6 carbon (δ 107.1) clearly indicated a polyalcohol linking the A-ring of flavan-3-ol unit.

A long-term reflux of **19** in ethanol containing 20% acetic[29] followed by repeated column chromatography over Sephadex LH-20, yielded, among many uncharacterized products, a crystalline compound, which seemed to be identical with (+)-gallocatechin (**30**).

To determine the linkage between (+)-gallocatechin (**30**) and castalin (**10**) moieties, the differentiation of favan-3-ol C-6 from C-8 substituted methyl ethers by ^{13}C NMR spectroscopy was applied. Methylation of **l9** with dimethyl sulfate and anhydrous potassium carbonate in dry acetone furnished the tetradecamethylate (**19a**), the FAB-MS of which exhibited a prominent $[M+H]^+$ peak at *m/z* 1117 consistent with the methylate of the proposed structure. The DEPT spectrum of **19a** showed an unsubstituted A-ring carbon signal at δ 89.5, the chemical shift being in good agreement with those of C-8 substituted (+)-catechin (**31**) derivative (gambiriin A_1 nonamethyl ether: δ 88.6, C-6), rather than those of the C-6 substituted alternative (gambiriin A_3 nonamethyl ether: δ 96.1, C-8)[30]. Based on these observation, in compound **19**, the castalin unit connected with the C-8 position of the (+)-gallocatechin nucleus by a carbon-carbon linkage.

The circular dichroism (CD) spectrum of **19** showed an intense positive cotton effect at 237 nm and a negative one at 263 nm, both corresponding well to those found in acutissimin C (**29**) whose triphenoyl (TP) ester moiety had been established to possess the *S,S*-configuration.[27] Thus, the atrop-isomerism in the triphenoyl group of **19** was concluded to be *S,S*-series.

Unequivocal structural assignment of **19** was successfully achieved by condensation of (+)-gallocatechin (**30**) and castalin (**10**).

Refluxing of the mixture in dry dioxane containing *p*-toluenesulfonic acid, followed by repeated column chromatography over Sephadex LH-20 with 60 % methanol, afforded a condensation product, which was found to be identical with **19** (Chart 1).

Therefore, the structure of compound **19** may be proposed as shown structure **19**, and named catappanin A.

Compound **28**, a pale brown amorphous power, with the ferric chloride reagent gave a dark blue coloration and in negative FAB-MS spectrum it exhibited the $[M-H]^-$ ion peak at *m/z* 497. In ^{1}H NMR spectrum, an aromatic signal appeared at δ 7.17 (4H, s) and the ^{13}C NMR data [δ 109.4, 109.9 (galloyl C-2, 2', 6, 6'), 121.2, 121.5 (galloyl C-1, 1'), 138.9, 139.1 (galloyl C-4, 4'), 145.9, 146.0 (galloyl C-3, 3', 5, 5'), 167.4 (-COO- x2)] indicated the presence of two galloyl groups in **28**. In addition, the ^{13}C NMR spectrum of **28** shows a six carbon sugar unit [δ 104.8 (C-1), 69.6, 72.8, 74.8, 78.7 (C-2, 3, 4, 5) and 64.4 (C-6)] together with a methoxyl signal (δ 57.2), suggesting the presence of a methyl hexoside moiety which was further supported by the observation of the signals at δ 3.51 (3H, s, OMe) and δ 4.45 (1H, d, *J*=8Hz, H-1) in the ^{1}H NMR spectrum.

Tannase hydrolysis of **28** yielded gallic acid (**1**), and 0.5 N NaOH hydrolysis of **28** gave a colorless hydrolysate, mp 110-112 °C, $[\alpha]_D$ -33.0° (H_2O), which was identified as methyl β-D-glucoside by comparison of the physical and spectral data with those of an authentic sample.

Analysis of the ^{1}H-^{1}H COSY spectrum of **28** and comparing with those of methyl β-D-glucoside revealed that relatively low field signals belong to the H-3 (δ 5.21, t, *J*=9 Hz) and H-6 (δ 4.41, dd, *J*=4, 11 Hz; δ 4.65, d, *J*=11 Hz) of acylated sugar moiety. The result indicated the galloyl groups were linked to the OH groups of a methyl glucoside at C-3 and C-6 position.

On the basis of spectroscopic and these chemical data, the structure of **28** was identified as methyl 3,6-di-*O*-galloyl-β-D-glucoside.

EXPERIMENTAL SECTION

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. ^{1}H and ^{13}C NMR spectra were taken with a JEOL FX-100 spectrometer with tetramethylsilane as an internal standard; chemical shifts are given on a δ (ppm) scale. FAB-MS was recorded on a JEOL JMS DX-300 spectrometer. Column chromatography was carried out with Sephadex LH-20 (25-100 , Pharmacia Fine Chemical), MCI-gel CHP20P (75-150, Mitsubishi Chemical Industries), Fuji-Gel ODS-G3 (43-65, Fuji Gel Hanbai), Bondapak C_{18}/porasil B (37-75 mesh, Waters) and Prep-pak 500/C_{18} (Waters). Thin-layer chromatography (TLC) was performed on precoated Kieselgel 60 F_{254} plates (0.2 mm; Merck) with solvent systems of benzene-ethyl formate-formic acid (1:7:1, 2:10:3); the spots were located by ultraviolet illumination and by spraying 3 % ferric chloride reagent or 10 % sulfuric acid followed by heating.

Plant Material

The dried bark of *Terminalia catappa* L. was collected in Ping-Tung, Taiwan, (July, 1996), and verified by Dr. Feng-Chi Ho (Taiwan Forestry Research Institute, Heng-Chun Branch, Ping-Tung, Taiwan.). The dried bark of *Terminalia parviflora* Presl. was collected in Sri Lanka (May,1995), and verified by Prof. Yukihiro Shoyama (Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan). The specimens were kept at the School of Pharmacy, Taipei Medical College, Taipei, Taiwan.

Extraction and Isolation

The dried bark of *Terminalia catappa* L. (5.1 Kg) was extracted four times with 80% aqueous acetone (10 L) at room temperature. The extract was concentrated under reduced pressure and the resulting brown precipitates

were removed by filtration. The filtrate was concentrated and subjected to Sephadex LH-20 column chromatography with water containing increasing amounts of MeOH and finally with a mixture of water-acetone (1:1) to give fraction 1 (185 g), fraction 2 (43 g), fraction 3 (71 g), and fraction 4 (86 g). Fraction 1 was chromatographed over Sephadex LH-20 and MCI-gel CHP20P (H_2O-MeOH) (1:0-0:1) to yield ellagic acid (**2**) (3.9 g) and 3-methoxy-4-hydroxyphenol-1-*O*-β-D-(6'-*O*-galloyl)-glucoside (**11**) (41 mg). Repeated chromatography of fraction 2 on Sephadex LH-20 (EtOH), MCI-gel CHP20P, Fuji-gel ODS-G3, and Bondapak C_{18}/porasil B (H_2O-MeOH) (1:0-0:1) yielded 2,3-(*S*)-HHDP-D-glucose (**3**) (190 mg), grandinin (**9**) (62 mg), castalin (**10**) (84 mg), 3,5-dimethoxy-4-hydroxyphenol-1-*O*-β-D-(6'-*O*-galloyl)-glucoside (**12**) (31 mg), and catappanin A (**19**) (375 mg) were isolated. Fraction 3 was chromatographed over Sephadex LH-20 (60 % MeOH, EtOH), Fuji-gel ODS-G3 and MCI-gel CHP20P, eluted with H_2O-MeOH (1:0-0:1) to give gallic acid (**1**) (2.3 g), punicalagin (**4**) (8.5 g), corilagin (**5**) (451 mg), tercatain (**6**) (1.2 g), castalagin (**8**) (9.1 g), acutissimin A (**17**) (74 mg), and eugenigranidin A (**18**) (106 mg). Fraction 4 was applied to columns of Sephadex LH-20, MIC-gel CHP20P, Fuji-gel ODS-G3, Bondapak C_{18}/porasil B (H_2O-MeOH) (1:0-0:1) and Sephadex LH-20 (EtOH) to give casuarinin (**7**) (78 mg), (-)-epicatechin 3-*O*-gallate (**13**) (162 mg), (-)-epigallocatechin 3-*O*-gallate (**14**) (1.2 g), procyanidin B-1 (**15**) (51 mg), and 3'-*O*-galloyl procyanidin B-2 (**16**) (465 mg). The dried bark of *Terminalia parviflora* Presl. (0.5 kg) was extracted four times with 80% aqueous acetone (2 L) at room temperature. The extract was concentrated under reduced pressure, and the resulting brown precipitates were removed by filtration. The filtrate was concentrated and subjected to Sephadex LH-20 column chromatography with water containing increasing amounts of MeOH and finally with a mixture of water-acetone (1:1) to give fraction 1 (18 g), fraction 2 (20 g), fraction 3 (43 g) and fraction 4 (26 g). Fraction 1 was chromatographed over MCI-gel CHP 20P, Fuji-gel ODS-G3 and Sephadex LH-20 (H_2O-MeOH) (1:0-0:1) to yield gallic acid (**1**) (223 mg), castalin (**10**) (470 mg), 2,3-(*S*)-HHDP-D-glucose (**3**) (75 mg). Fraction 2 was chromatographed over MCI-gel CHP20P and Fuji-gel ODS-G3, eluted with H_2O-MeOH (1:0-0:1) to give grandinin (**9**) (530 mg). Repeated chromatography of fraction 3 on MCI-gel CHP20P, Fuji-gel ODS-G3, Bondapak C_{18}/porasil B, Prep-pak 500/C_{18} and Sephadex LH-20, eluted with H_2O-MeOH (1:0-0:1), yielded punicalin (**26**) (101 mg), 2,3-(*S*)-HHDP-6-*O*-galloyl-D-glucose (**24**) (82 mg), castalagin (**8**) (17 g), and 2-*O*-galloyl punicalin (**27**) (16 mg). Fraction 4 was applied to columns of Fuji-gel ODS-G3, MCI-gel CHP20P, Bondapak C_{18}/porasil B, Prep-pak 500/C_{18} and Sephadex LH-20, eluted with H_2O-MeOH (1:0-0:1) to yield 1,6-di-*O*-galloyl-β-D-glucose (**21**) (74 mg) castamollinin (**25**) (2.7 g), punicalagin (**4**) (2.5 g), flavogallonic acid (**20**) (147 mg), ellagic acid (**2**) (830 mg), 3,6-di-*O*-galloyl-D-glucose (**22**) (36 mg), 1,3,6-tri-*O*-galloyl-β-D-glucose(**23**) (74 mg) and methyl 3,6-di-*O*-galloyl-β-D-glucoside (**28**) (65 mg).

Catappanin A (19)

A pale brown amorphous powder, $[\alpha]]_D^{20}$ +15.2° (*c*=1.2, MeOH); Anal. Calcd for $C_{42}H_{32}O_{24}$ 3/2 H_2O: C, 53.23; H, 3.72. Found: C, 53.28; H, 3.73; Negative FAB-MS *m/z*: 919 $[M-H]^-$; ^1H NMR (acetone-d_6-D_2O) (100 MHz) δ 2.55 (1H, dd, *J*=16, 8 Hz, gallocatechin (gc.) H-4), 2.87 (1H, dd, *J*=16, 4 Hz, gc. H-4), 3.85-4.02 (4H, m, H-4, 6 and gc. H-3), 4.54-4.65 (2H, m, H-3 and gc. H-2), 4.58 (1H, br s, H-1), 5.25 (1H, m, H-5), 5.30 (1H, br s, H-2), 6.06 (1H, s, gc. H-6), 6.48 (2H, s, gc. H-2', 6'), 6.75 (1H, s, TP-H); ^{13}C NMR (acetone-d_6-D_2O) (25.05 MHz) δ 27.6 (gc. C-4), 38.1 (C-1), 62.4 (C-6), 68.1 (gc. C-3), 70.2, 74.4, 74.8, 77.2 (C-2, 3, 4, 5), 82,1 (gc. C-2), 96.6 (gc. C-6), 100.2 (gc. C-4a), 105.7 (gc. C-8), 107.1 (gc. C-2', 6'), 108.9 (TP C-6"), 113.5, 113.8, 114.2 (TP C-2, 2', 6'), 116.6 (TP C-2"), 121.7

(TP C-6), 126.3, 126.5 128.6 (TP C-1, 1', 1"), 131.6, 133.1 (gc. C-1', 4'), 135.1, 136.1, 136.8 (TP C-4, 4', 4"), 143.3, 144.2, 144.6, 144.7, 145.4 (TP C-3, 5, 3', 5', 3 ", 5"), 145.9 (gc. C-3', 5'), 154.0, 155.3, 156.4 (gc. C-5, 7, 8a), 166.4, 164.3 (-COO-); CD (MeOH): $[\theta]_{245}$ +10.1 ^{4}, $[\theta]_{265}$ -2.1x10^4 and $[\theta]_{315}$ +2.1xl0^4; Acid-catalyzed degradation of **19**: A solution of **19** (120 mg) in ethanol (7 ml) and acetic acid (2.0 ml) was heated under reflux for 5 days. The solvent was evaporated off under reduced pressure, and the residue was chromatographed over Sephadex LH-20 with ethanol to yield (+)-gallocatechin (**30**) as colorless needles (2.5 mg), mp 172-175 °C, $[\alpha]_D^{16}$ +12.4° (*c*=1.0, acetone), silica gel TLC, R_f=0.56 (benzene-ethyl formate-formic acid, 1:7:1). Synthesis of catappanin A methylate (**19a**): A mixture of **19** (238 mg), dimethyl sulfate (3 mL), and anhydrous potassium carbonate (2.0 g) in dry acetone (15 ml) was heated under reflux for 3.5 h. After removal of the inorganic salts by filtration, the filtrate was concentrated under reduced pressure and subjected to silica gel chromatography. Stepwise elution with benzene containing increasing proportions of acetone furnished **19a** (55 mg) as a white amorphous powder, $[\alpha]_D^{20}$ +26.4° (*c*=1.5, $CHCI_3$); Positive FAB-MS *m/z*: 1117 $[M+H]^+$; ^{1}H NMR ($CDCI_3$) (100 MHz) δ 2.57 (1H, dd, *J*=16, 10 Hz, gc. H-4), 3.17 (1H, dd, *J*=16, 6 Hz, gc. H-4), 3.46, 3.52, 3.55, 3.57, 3.67, 3.79, 3.82, 3.84, 3.92, 3.95, 4.07, 4.17 (each 3H, s, OMe), 3.97 (6H, s, OMe), 3.80-4.00 (m, H-4, 6 and gc. H-3, overlapped with OMe signals), 4.47-4.72 (2H in total, m, H-3, gc. H-2), 4.64 (1H, br s, H-1), 5.25 (1H, br s, H-2), 5.41 (1H, m, H-5), 6.08 (1H, s, gc. H-6), 6.93 (2H, s, gc. H-2', 6'), 6.97 (1H, s, TP-H); ^{13}C NMR ($CDCl_3$) (67.80 MHz) δ 26.7 (gc. C-4), 37.1 (C-1), 55.5, 61.0, 61.2, 61.3, 61.7 (OMe), 62.7 (C-6), 65.5 (C-4), 68.8 (gc. C-3), 70.2, 71.9, 73.3 (C-2, 3, 5), 76.2 (gc. C-2), 89.2 (g.c. C-6), 157.9, 158.7 (gc. C-5, 7, 8a), 164.4, 164.6, 165.7 (-COO-). Synthesis of **19**: A mixture of (+)-gallocatechin (**30**) (200 mg) and castalin (**10**) (200 mg) in dry dioxane (10 ml) containing *p*-toluenesulfonic acid (15 mg) was heated under reflux for 3 h with stirring. The solvent was evaporated off under reduced pressure and the residue was chromatographed over Sephadex LH-20 with EtOH containing increasing amounts of water-acetone (1:1) and then over Bondapak C_{18}/porasil B with water containing increasing amounts of MeOH, to yield a condensation product (8 mg), which was identified as **19** by $[\alpha]_D$ and ^{1}H and ^{13}C NMR comparisons.

Methyl 3,6-di-*O*-gallo-β-D-glucoside (28)

A pale brown amorphous powder, $[\alpha]_D^{20}$ +24.1° (*c*=0.8, acetone+H_2O); Anal. Calcd for $C_{21}H_{22}O_{14}$.1/2H_2O: C, 49.71; H, 4.57. Found: C, 49.57; H, 4.80; Negative FAB-MS *m/z*: 497 $[M-H]^-$; ^{1}H NMR (acetone-d_6+D_2O) δ 3.51 (3H, s, OMe), 4.41 (1H, dd, *J*=11, 4 Hz, H-6), 4.45 (1H, d, *J*=8 Hz, H-1), 4.65 (1H, d, *J*=11 Hz, H-6), 5.21 (1H, t, *J*=9 Hz, H-3), 7.17 (4H, s, galloyl H); ^{13}C NMR (acetone-d_6-D_2O) δ 57.2 (OMe), 64.4 (C-6), 69.6, 72.8, 74.8, 78.7 (C-2, 3, 4, 5), 104.8 (C-1), 109.4, 109.9 (galloyl C-2, 6), 121.2, 121.5 (galloyl C-1), 138.9, 139.1 (galloyl C-4), 145.9, 146.0 (galloyl C-3, 5), 167.4 (2C, -COO-). Tannase hydrolysis of **28**: A solution of **28** (3 mg) in H_2O was shaken with tannase at room temperature for 2 h. Gallic acid (**1**) was detected on TLC [solvent: $CHCl_3$-MeOH-H_2O (7:3:0.5), R_f 0.28] of the reaction mixture as a sole product positive to the ferric chloride reagent. 0.5 N NaOH alkaline hydrolysis of **28**: A solution of **28** (15 mg) in 0.5 N aqueous NaOH (1 mL) at room temperature for 2 h. After neutralization with Amberlite IR-120 B (H^+ form), the solution was concentrated *in vacuo* and the residue was chromatographed over Sephadex LH-20 using EtOH to yield methy β-D-glucoside (4 mg), colorless plates (MeOH), mp 110-112 °C, $[\alpha]_D$ -32° (*c*=1.2, H_2O); IR (KBr) cm^{-1}: 3340, 2850, 1448, 1401, 1220, 1095, 1079, 1030, 992, 884, and identified with methyl β-D-glucoside by comparison of silica gel TLC, R_f=0.15 (benzene-ethyl formate-formic acid; 2:7:1) with that of an authentic sample.

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