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Studies on *Cerbera*. I. Cardiac Glycosides in the Seeds, Bark, and Leaves of *Cerbera manghas* L.

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Cardenolides in the seeds, bark, and leaves of *Cerbera manghas* L. were investigated. From the seeds, tanghinigenin glycosides were isolated along with known digitoxigenin glycosides, cerberin, neriifolin, thevetin B, and 2′-O-acetyl thevetin B. From the barks of root and stem, gentiobiosyl thevetoside, glucosyl thevetoside, and thevetoside of tanghinigenin and 17βH-tanghinigenin were obtained. The cardenolides in the air-dried leaves were found to vary with seasons. 17βH-neriifolin is major and preferable to neriifolin in July, while 17βH-deacetyltanghinin and deacetyltanghinin present in the leaves of Feb.

Keywords——*Cerbera manghas*; Apocynaceae; cardenolides; cardiac glycosides; 17βH-cardenolides; polyamide column chromatography

Several species in genus *Cerbera*(Apocynaceae) grow widely in the areas of South-East Asia and Pan-Indian Ocean. In Japan, *Cerbera manghas* L. is indigenous in Ryukyu Islands, being known as "Okinawa-kyotikuto" or "Mifukuragi." While *C. manghas* is described as synonym of *C. odollam* GAERTN. in Index Kewensis, the both plants have been divided into different species by some taxonomists.[2] Bisset[3] attempted to resolve this problem by comparison of the cardenolides in the seeds and bark of these plants with an aid of paper chromatography. Concerning the isolation of the cardiac glycosides, Matsubara[4] obtained "cerberin," toxic constituent in the seeds of Mifukuragi. Rangaswami, Venkata Rao, and associates isolated cerberin, neriifolin, thevetin B,[5a] 2′-O-acetyl thevetin B,[5b] and 2′,4′-di-O-acetyl neriifolin[5c] from the seeds of *C. odollam*. Australian group[6] reported the isolation and structure elucidation of cerbertin and cerbertatin from *C. floribunda* and *C. dilata*. This paper deals with the cardenolides in the kernels, the bark, and the leaves of *C. manghas* in Okinawa Island.

The kernels, collected in February of 1974, were homogenized with methanol and the concentrate of methanolic solution was partitioned with hexane, benzene, chloroform, and with butanol. In these fractions, were observed eight spots, A-1—A-8, in order of decreasing polarity, on thin-layer chromatography (TLC), and A-3, -4, -7, and -8 were major (Fig. 1).

A-7 and A-8 were respectively identified as neriifolin (=digitoxigenin β-L-thevetoside)[7] and cerberin (=2′-O-acetyl neriifolin). During chromatographic purification of neriifolin, a small amount of another compound showing orange fluorescence with $SbCl_3$ reagent, and similar *Rf* value as that of A-7, was isolated (B-7). The physical constants were in good

1) Location: *Nanakuma 11, Nishi-ku, Fukuoka*; *a*) To whom inquiries should be addressed.
2) R.C. Bakhizen van den Brink, Jr., "Notes on the Flora of Java VI," *Blumea*, **6**, 386 (1950); and *cf.* ref. 3.
3) N.G. Bisset, *Annales Bogorienses*, **4** (Part 2), 153 (1961).
4) T. Matsubara, *Nippon Kagakukaishi*, **58**, 1104, 1195, 1201, 1230, 1237, 1239 (1930).
5) *a*) S. Rangaswami and E. Venkata Rao, *J. Sci. Ind. Res.* (India), **16B**, 209 (1957); *b*) E. Venkata Rao, B.S. Sastry, and M.N. Narayanan, *Indian J. Pharm.*, **36**, 75 (1974); *c*) E. Venkata Rao and M. Appa Rao, *Phytochemistry*, **15**, 848 (1976).
6) J. Cable, R.G. Coombe, and T.R. Watson, *Aust. J. Chem.*, **17**, 1423 (1964); *idem*, *ibid.*, **18**, 1080 (1965).
7) Linkage of L-thevetose to digitoxigenin was assigned as β by Voigtländer, *et al.*[8] according to the coupling constant of anomeric protons in neriifolin (δ=4.87, J=7 Hz) and in cerberin (δ=5.05, J=4 Hz).
8) H.W. Voigtländer, G. Balsam, and G. Herbst, *Arch. Pharm. Ber. Dtsch. Pharm. Ges.*, **302**, 538 (1969).

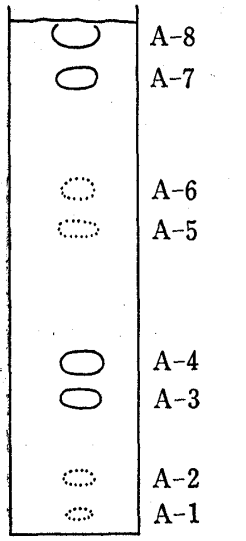
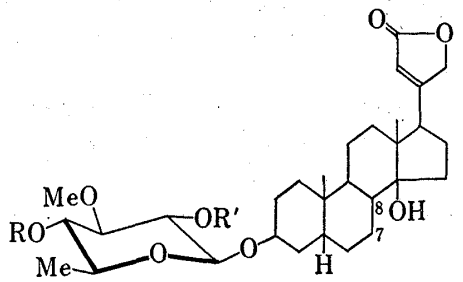
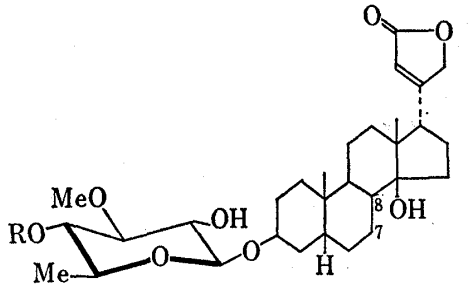


Fig. 1. Thin-Layer Chromatogram of the Cardenolides of the Seeds



A–3 (=thevetin B): R=β-gentiobiosyl, R′=H
A–4 (=2′-O-Ac-thevetin B): R=β-gentiobiosyl, R′=Ac
A–7 (=neriifolin): R=R′=H
A–8 (=cerberin): R=H, R′=Ac
B–3: R=β-gentiobiosyl, R′=H, $C_{7,8}\beta\beta$-epoxy
B–5: R=β-D-glucosyl, R′=H, $C_{7,8}\beta\beta$-epoxy
B–7 (=deacetyltanghinin): R=R′=H, $C_{7,8}\beta\beta$-epoxy



C–7 (=17βH-neriifolin): R=H
D–5: R=β-D-glucosyl, $C_{7,8}$ $\beta\beta$-epoxy
D–7 (=17βH-deacetyltanghinin): R=H, $C_{7,8}\beta\beta$-epoxy

Chart 1

agreement with those of deacetyltanghinin,[9] and IR spectra of the both compounds were superimposable. The linkage of L-thevetose was assigned β as in the case of neriifolin and cerberin, according to the anomeric proton, centered at δ 5.08 with a spacing of 4 Hz in B-7 acetate.

By means of polyamide (PA) and silica gel (SG) column chromatography of the butanol layer, two polar glycosides, A-3 and A-4 were obtained as crystals. From the facts that both A-3 and A-4 formed the identical acetate which, on acetolysis, gave gentiobiose acetate, and that A-4 was hydrolyzed to A-8 with β-glucosidase of *Thevetia* seeds,[10] as well as their physical constants, they were determined as thevetin B and its 2′-O-acetate, respectively. On the hydrolyses of A-3 and A-4, were obtained partially hydrolyzed compounds, which were coincided with A-5 and A-6, respectively.

B-3, showing similar polarity as that of A-3 on TLC and the same colour reaction as of B-7, was isolated on successive PA chromatography of the mother liqour portion of thevetin B. On enzymic hydrolysis, B-3 afforded B-7 and B-5, the latter appeared to be deglucosyl derivative of B-3 (=glucosyl B-7), by comparison of its polarity with A-5. When B-5 acetate was acetolyzed, one of the product was identical by gas chromatography (GLC) with β-D-glucosyl-(1′→4)-L-thevetose acetate, prepared from A-5 acetate, and gentiobiose acetate was formed by acetolysis of B-3 acetate. B-3 therefore was found to be β-gentiobiosyl-(1″→4′)-deacetyltanghinin.

9) *a*) H. Helfenberger and T. Reichstein, *Helv. Chim. Acta*, **35**, 1503 (1952); *b*) H.P. Sigg, C. Tamm, and T. Reichstein, *Helv. Chim. Acta*, **38**, 166 (1955); E. Flury, E. Weiss, and T. Reichstein, *Helv. Chim. Acta*, **48**, 1113 (1965).
10) H. Helfenberger and T. Reichstein, *Helv. Chim. Acta*, **31**, 1470 (1948).

A-1 and A-2 were suggested to be the glycosides having one additional glucose unit to A-3 and A-4, respectively, although enough amounts for further investigation were not obtained.

Powdered dried stem and root barks, herbolized in Feb. of 1974, were treated principally in the same manner as in the seeds, and three spots, corresponding to A-3, -5, and -7, on TLC, were detected in the extract of both the stem and the root barks. According to the insufficiency of material, subsequent studies were carried out using the mixture of the extracts of the root and stem barks.

The least polar and minor spot in the three exhibited the same *Rf* value and staining as those of B-7, and confirmed the structure as deacetyltanghinin after acetylation. In the mother liquor fraction of B-7, another new cardenolide (D-7) was detected and assumed to be epoxycardenolide with 17βH-configuration according to NMR spectroscopy. The structure was elucidated as 17βH-tanghinigenin β-L-thevetoside (=deglucosyl D-5) by direct comparison with the sample, prepared from enzymic hydrolysis of D-5. The spot corresponding to A-5 was found, after acetylation, to be a mixture of two compounds (B-5 and D-5) and the each acetate was separated by crystallization following column chromatography. One of them, B-5 acetate was determined by comparison with the sample obtained from B-3 by enzymic hydrolysis. Another compound, D-5 acetate, indicating purple colour with $SbCl_3$ reagent, was suggested to be 17βH-derivative of A-5 or B-5. When B-5 acetate was heated with sodium tosylate (NaOTs) and sodium acetate (NaOAc) in dimethylformamide (DMF),[11] the product was identical with D-5 acetate in any respects. Thus, D-5 was determined as 17βH-tanghinigenin β-D-glucosyl-(1''$\rightarrow$4')-β-L-thevetoside. The spot corresponding to A-3 in the most polar fraction was identified as B-3.

In order to compare the cardenolides of the leaves with those of the seeds and the bark, the leaves collected in the four seasons in 1974 were investigated (Table I). From the air-dried leaves of Feb., B-7 and D-7 were obtained, along with a small amount of A-7, while in the other three seasons, a new compound (C-7) was obtained. C-7 exhibited no resonance at 2.5—3.0 ppm, due to C-17αH. According to the fact that C-7 was accompanied by A-7, in the leaves of May, July, and Nov., C-7 was presumed to be 17βH-derivative of neriifolin. The isomerization reaction[11] of A-7 furnished 17βH-neriifolin, physical properties of which were in good agreement with those of C-7 and then, the structure of C-7 was established.

Table I. Cardenolides of the Leaves in the Four Seasons (%)

	May	July	Nov.	Feb.
Digitoxigenin β-L-thevetoside	0.01	0.01	0.004	Trace
17βH-Digitoxigenin β-L-thevetoside	0.02	0.07	0.01	±
Tanghinigenin β-L-thevetoside	±	±	Trace	0.007
17βH-Tanghinigenin β-L-thevetoside	±	±	±	0.003

Tanghinigenin glycosides have been known as the major cardenolides in the seeds of *Tanghinia venenifera*.[9] The isolation of tanghinigenin (B-series) and 17βH-tanghinigenin (D-series) glycosides from *C. manghas* suggests chemotaxonomically close relationship between *Tanghinia* and *Cerbera*. Since several 17βH-cardenolides have been found from natural sources, some plants are believed to possess isomerization enzyme system from 17αH- to 17βH-cardenolides.[12] Whereas normal digitoxigenin glycosides are abundant in nature, 17βH-digitoxigenin is very rare,[13] and the occurrence of 17βH-tanghinigenin glycosides is the first example. It is noteworthy that the biosides of tanghinigenin and its 17βH-isomer are the major cardenolides in the bark, while the trioside of digitoxigenin in the seeds, and the

11) J.H. Russel, O. Schindler, and T. Reichstein, *Helv. Chim. Acta*, 43, 167 (1960).
12) B. Singh and R.P. Rastogi, *Phytochemistry*, 9, 315 (1970).
13) M. Frerejacque, *C.R. Acad. Sci.*, 248, 3027 (1959).

monosides of the four cardenolides in the air-dried leaves, and also that the ratio of digitoxigenin, tanghinigenin, and their 17βH-isomers in the leaves varies unequivocally in the seasons. The external or internal factor of 7,8-epoxidation and 17-isomerization is to be investigated.

Experimental

Melting point was measured on Kofler block and uncorrected. NMR spectra were run in $CDCl_3$ solution with internal TMS using Hitachi R-22 Spectrometer. On column chromatography, polyamide (PA) was purchased from Wako Chemicals Co., Ltd. and eluted with water and water–MeOH. Silica gel (SG) column chromatography was carried out using Merck silica gel and eluted with $CHCl_3$–MeOH–water (7: 2: 1.2—0.7, 7: 3: 1.2—0.7, bottom layer, Solv. 1) or benzene–acetone (Solv. 2) system.

Extraction and Isolation of Cardiac Glycosides in the Seeds——Seeds (20 kg) were collected in Feb. of 1974, at Kunigami-son of Okinawa Island, and the hard shell was removed. The kernels were homogenized with MeOH and the mixture was filtered. The filtrate was concentrated *in vacuo* to 500 ml, diluted with a same volume of water and partitioned successively with hexane, benzene, $CHCl_3$, and with BuOH. Benzene and $CHCl_3$ extracts (3.5 g and 2 g, respectively) were combined together and chromatographed on SG column. A-7 and A-8 were eluted with Solv. 2.

Neriifolin (A-7)——A-7 was crystallized from EtOAc–hexane to give needles (240 mg), mp 224—227°, $[\alpha]_D^{20}$ −37.2° (c=0.19, $CHCl_3$), (neriifolin: mp 226—230°, $[\alpha]_D$ −49.7°).[5a] On reflux with 0.5 N H_2SO_4–50% EtOH for 1 hr, anhydrodigitoxigenin (mp 200—232°) and L-thevetose were identified.

Cerberin (A-8)——A-8 was crystallized from EtOAc–hexane to give needles (400 mg), mp 218—221°, $[\alpha]_D^{25}$ −83.8° (c=0.17, $CHCl_3$), (cerberin: mp 212—216°, $[\alpha]_D$ −84.2°),[5a] NMR δ(ppm): 0.90, 0.98 (3H each, s, C-18 and -19), 1.30 (3H, d, J=6 Hz, C-6′), 2.08 (3H, s, C-2′-OAc), 2.80 (1H, m, C-17), 3.64 (3H, s, C-3′-OCH_3), 4.72 (1H, dd, J=4 and 10 Hz, C-2′), 5.00 (2H, dd, J=3 and 6 Hz, C-21), 5.12 (1H, d, J=4 Hz, C-1′), 5.97 (1H, bs, C-22). On saponification with 0.8% $KHCO_3$ in 80% MeOH at room temperature, A-7 was obtained, mp 219—221°.

Deacetyltanghinin (B-7)——Mother liquor fraction of A-7 was repeatedly chromatographed over SG with Solv. 2, followed by crystallization from hexane–acetone to give B-7 as prisms (40 mg), mp 213—216°, $[\alpha]_D^{25}$ −53.5° (c=0.43, MeOH). *Anal.* Calcd. for $C_{30}H_{44}O_9$: C, 65.67; H, 8.08. Found: C, 65.10; H, 8.20. (deacetyltanghinin: mp 217°[9a] or 238—241°,[9b] $[\alpha]_D$ −59°, acetate: mp 189°),[9] NMR (C_5H_5N): 1.00, 1.04 (3H each, s, C-18 and -19), 1.67 (3H, d, J=6 Hz, C-6′), 3.41 (1H, d, J=6 Hz, C-7), 3.86 (3H, s, C-3′-OCH_3), 5.11 (2H, d, J=8 Hz, C-21), 5.24 (1H, d, J=3 Hz, C-1′), 6.14 (1H, bs, C-22). B-7 Acetate was prepared in an usual manner, and crystallized from EtOAc–hexane, mp 187—197° (dec.), NMR: 0.90, 0.96 (3H, each, s, C-18 and -19), 1.08 (3H, d, J=6 Hz, C-6′), 2.02, 2.07 (3H each, s, C-2′, -4′-OAc), 3.20 (1H, d, J=5 Hz, C-7), 3.41 (3H, s, C-3′-OCH_3), 4.83 (2H, d, J=5 Hz, C-21), 5.08 (1H, d, J=4 Hz, C-1′), 5.82 (1H, bs, C-22).

Thevetin B (A-3)——The BuOH ext. (19.5 g) was passed through PA column and eluted with water. Fr. 1: A-1, A-2, A-3, A-4 (15 g), Fr. 2: A-3>A-4 (0.7 g), Fr. 3: A-3<A-4 (1.2 g), Fr. 4: A-4 (0.5 g), Fr. 5: A-5, A-6 (<50 mg). Fr. 1 was rechromatographed over SG with Solv. 1; Fr. 1′: A-4 (6.3 g), Fr. 2′: A-3 (5.5 g), Fr. 3′: A-1, A-2, A-3 (200 mg). Fr. 2′ was rechromatographed over PA and crystallized from water to give needles (1.2 g), mp 192—194°, $[\alpha]_D^{25}$ −53.1° (c=0.21, MeOH). Acetate was crystallized from MeOH–ether to give needles, mp 138—140°, $[\alpha]_D^{25}$ −55.3° (c=0.25, MeOH). *Anal.* Calcd. for $C_{58}H_{82}O_{26}\cdot H_2O$: C, 57.41; H, 6.98. Found: C, 57.75; H, 6.92. IR of A-3 acetate was superimposable with that of A-4 acetate and no melting point depression was observed on admixture of the both acetates. A-3 was hydrolyzed with β-glucosidase (prepared from *Aspergillus*)[14] to give A-5, which was acetylated without further purification. The acetate was crystallized from dil. MeOH to give needles, mp 155—158°, $[\alpha]_D^{25}$ −67.7° (c=0.12, MeOH), and was identical with A-6 acetate. A-3 Acetate was heated with Ac_2O and a small amount of $ZnCl_2$ at 100° for 30 min. The product was purified through SG column and crystallized from dil. EtOH to give α-gentiobiose acetate (mp 191—192°).

2′-O-Acetyl Thevetin B (A-4)——Fr. 1′ and Fr. 4 were crystallized from acetone–ether to give fine needles (A-4, 3.0 g), mp 225—235°, $[\alpha]_D^{25}$ −74.9° (c=0.20, MeOH). *Anal.* Calcd. for $C_{44}H_{68}O_{19}\cdot H_2O$: C, 57.50; H, 7.68. Found: C, 57.01; H, 7.60. A-4 (160 mg) was treated with β-glucosidase prepared from the seeds of *Thevetia neriifolia*,[10] followed by column chromatography to give A-8 (mp 234—237°, $[\alpha]_D^{25}$ −79.1° (c=0.10, $CHCl_3$)) and A-6 (mp 150—155°, $[\alpha]_D^{25}$ −76.6° (c=0.21, MeOH)). A-6 was subjected to acetylation, followed by crystallization from dil. MeOH to give needles, mp 159—162°, $[\alpha]_D^{25}$ −65.7° (c=0.21, MeOH). *Anal.* Calcd. for $C_{46}H_{66}O_{18}\cdot H_2O$: C, 59.73; H, 7.41. Found: C, 59.68; H, 7.35. On saponification of A-4 with 0.8% solution of $KHCO_3$ in 80% MeOH, A-3 (mp 195—200°) was obtained. On acetylation, A-4 acetate was obtained as needles, mp 140—144°, $[\alpha]_D^{25}$ −55.0° (c=0.24, MeOH).

Gentiobiosyl Deacetyltanghinin (B-3)——Mother liquor fraction of A-3 was successively passed through PA column to give homogeneous solid (300 mg), which exhibited yellow colour spot on TLC with $SbCl_3$ reagent. On acetylation, followed by crystallization from dil. MeOH, B-3 acetate was obtained as needles,

14) Enzyme was furnished from Amano Pharm. Co., Ltd. in Nagoya.

mp 134—136°, $[\alpha]_D^{25}$ −60.0° (c=0.10, MeOH). *Anal.* Calcd. for $C_{58}H_{80}O_{27}\cdot H_2O$: C, 56.75; H, 6.73. Found: C, 56.52; H, 6.77. B-3 (90 mg) was treated with β-glucosidase (*Aspergillus*) and the product was acetylated without further purification. The acetate was purified by SG column and crystallized from EtOAc–hexane to give B-5 acetate as needles, mp 144—147°, $[\alpha]_D^{25}$ −70.0° (c=0.50, MeOH). B-3 (80 mg) was treated with snail digestive juice and the product was crystallized, after chromatography, from EtOAc–hexane to give B-7 as needles, mp 210—215°. B-3 acetate (80 mg) was heated at 100° with Ac_2O and $ZnCl_2$ and the sugar acetate was purified through column to give α-gentiobiose acetate, mp 190—192°. B-5 acetate (50 mg) was subjected to acetolysis in the same manner as above, and the sugar acetate was coincided with that from A-5 acetate on TLC and GLC (t_R: 21.6 min, the biose acetate from A-5 acetate: 21.6 min, α-gentiobiose acetate: 34.7 min, 1.5% SE-52, 2.1 m, column temp.: 229°, N_2: 1.0 kg/cm²).

Extraction of Cardiac Glycosides in the Barks——Dried powdered root bark (1.9 kg), collected in Feb. of 1974, was percolated with MeOH. To the concentrated percolate, equal volume of water was added and the mixture was extracted with hexane (ext. 57.6 g), benzene (6.2 g), $CHCl_3$ (6.9 g) and with $CHCl_3$–EtOH (2: 1) (32.0 g). The percolate from 4 kg of the stem bark of the same tree was worked up in the same manner as that of the root bark; hexane (94.9 g), benzene (23.5 g), $CHCl_3$ (14.0 g), and $CHCl_3$–EtOH (2: 1) (65 g). The corresponding fractions of the root and stem barks were compared on TLC and were found to contain the same compounds. They were respectively combined together.

B-7 and 17βH-Tanghinigenin β-L-Thevetoside (D-7) from Benzene Extract——The benzene extract was crystallized from MeOH to separate yellow pigment[15] and the mother liquor fraction was subjected to SG column chromatography using Solv. 2. The fractions showing positive reaction by Kedde reagent and similar polarity as of A-7 were combined and acetylated by usual manner. The acetates were purified through SG column and separated to two acetates; B-7 acetate (mp 187—197°, *ca.* 60 mg) and a new amorphous acetate (D-7 acetate, *ca.* 50 mg). NMR of D-7 acetate: 1.00, 1.09 (3H each, s, C-18 and -19), 1.13 (3H, d, J=6 Hz, C-6′), 2.05, 2.10 (3H, each, C-2′-, -4′-OAc), 3.24 (1H, d, J=7 Hz, C-7), 3.44 (3H, s, C-3′-OCH_3), 4.77 (2H, bs, C-21), 5.08 (1H, d, J=4 Hz, C-1′), 5.89 (1H, s, C-22). The acetates were deacetylated with 0.8% $KHCO_3$ in 80% MeOH to give B-7 (mp 227—232°) and D-7 (mp 216—222°, crystallized from MeOH). D-7: *Anal.* Calcd. for $C_{30}H_{44}O_9$: C, 65.67; H, 8.08. Found: C, 65.87; H, 8.25. On the comparison with D-7, prepared from D-5 with snail digestive juice, both samples were identical.

B-5 and 17βH-Tanghinigenin β-D-Glucosyl-(1″→4′)-β-L-thevetoside (D-5)——The $CHCl_3$ fraction was subjected successively to PA and SG column chromatographies and the crude cardenolides fractions combined, were acetylated. Two acetates were finally separated by SG column using Solv. 2. B-5 acetate was crystallized from EtOAc–hexane to give needles (mp 143—146°, *ca.* 100 mg). D-5 acetate was crystallized from EtOAc–hexane to give needles, mp 218—221°, $[\alpha]_D^{25}$ −62.0° (c=0.10, $CHCl_3$). D-5 acetate was saponified with 0.8% $KHCO_3$ in 80% MeOH to give D-5 as solid. On enzymic hydrolysis of D-5 with snail digestive juice, D-7 was obtained and crystallized from EtOAc–hexane as needles, mp 214—219°, IR of which was superimposable with that of D-7, obtained from the benzene ext. B-5 acetate (200 mg) was heated in DMF (15 ml) with NaOTs (700 mg) and NaOAc (300 mg) for 13 hr at 110°. The product was purified by column chromatography and crystallization from EtOAc–hexane to give 17 mg of needles, mp 217—219°, and identified as D-5 acetate (mp, IR).

Isolation of B-3 from $CHCl_3$–EtOH (2: 1) Fraction——After the purification of the extract by PA column with water elution and SG column with Solv. 1 elution, the fractions of the cardenolides were subjected to acetylation and resulting acetate was again purified through SG column. On recrystallization from dil. MeOH, B-3 acetate was obtained as prisms, mp 134—136° (*ca.* 50 mg).

Extraction and Isolation of the Cardenolides in the Leaves——Each crop of the leaves, herbolized in Feb. (dried weight: 2.2 kg), May (1.8 kg), July (4.2 kg), and Nov. (5.5 kg) of 1974, at the same district as of the seeds and the barks, was dried in the shade, powdered, and percolated with MeOH. The percolates were concentrated, diluted with water and extracted with hexane and $CHCl_3$. The isolation of the cardenolides was conducted in the same manner as in the seeds and the bark. Yields in the each season were indicated in Table I.

17βH-Digitoxigenin β-L-Thevetoside (C-7)——C-7 was crystallized from EtOAc–hexane to give prisms, mp 242—245°, $[\alpha]_D^{25}$ −42.0° (c=0.10, MeOH). *Anal.* Calcd. for $C_{30}H_{46}O_8$: C, 67.38; H, 8.67. Found: C, 67.09; H, 8.89. NMR: 1.00, 1.03 (3H each, s, C-18 and -19), 1.30 (3H, d, J=6 Hz, C-6′), 3.79 (3H, s, C-3′-OCH_3), 4.74 (2H, bs, C-21), 4.84 (1H, d, J=4 Hz, C-1′), 5.87 (1H, s, C-22). A-7 (260 mg) was heated in DMF (20 ml) with NaOTs (990 mg) and NaOAc (400 mg) for 13 hr at 110°. The product, after purification by column chromatography, was crystallized from EtOAc–hexane to give 42 mg of prisms, mp 239—242°, whose IR was in good agreement with that of C-7.

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