## Investigation of the Extracted Species

In the previous paper,[10] the colors of the extracts into 1,2-dichloroethane were classified into three categories; (1) blue extracts which are observed in the presence of the quaternary ammonium salts (2) red extracts which are developed in the presence of alkaloids (3) yellow extracts which are the same color as the reagent blank. In this paper, the extracts into 1,2-dichloroethane or toluene are classified into four categories by electric conductivity in Table I; (1) blue; dissociated ion-pair complexes, *e.g.*, berberine, benzethonium, sparteine in 1,2-dichloroethane (2) blue; associated ion-pair compounds, *e.g.*, berberine in toluene (3) red; associated charge transfer complexes, *e.g.*, ephedrine, quinine ethylcarbonate, procaine in 1,2-dichloroethane or sparteine in toluene (4) yellow; associated H·TBPE. These phenomena can be attributed to the complicated interactions between the cation and TBPE which are sensitively influenced by the molecular structure of the cation and the dielectric nature of solvents. Consequently, the extracted species may be formulated as [quinine ethylcarbonate]·H·[TBPE] from continuous variation plots.

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10) T. Sakai, I. Hara, and M. Tsubouchi, *Chem. Pharm. Bull.* (Tokyo), **24**, 1254 (1976).

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# Studies on the Constituents of *Mallotus japonicus* Muell. Arg. II.[1] A Corotoxigenin Trioside from the Seeds[1]

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A new cardiac trioside was isolated from the seeds of *Mallotus japonicus* Muell. Arg. (Euphorbiaceae) and it was identified as corotoxigenin β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside.

In the preceding paper, the authors reported isolation and characterization of α-L-rhamnopyranosides and β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosides of corotoxigenin, mallogenin, coroglaucigenin and panogenin, aglycones common to those in *Mallotus philippinensis* and *M. paniculatus*.[3] The authors mentioned that the glucorhamnosides were the genuine cardiac glycosides. However, one more polar compound sensitive to the Kedde reaction was detected on closer examination of the intermediate emulsion layer which formed on butanol extraction of water layer after a series of solvent extraction. This paper deals with characterization of this newly detected cardiac glycoside (I).

The emulsion layer was separated and evaporated to a dark brown powder and the aqueous solution was passed through the polyamide column. The eluate was colorless but I could not be separated from other polar contaminants. Silica gel column chromatography of the

1) Part I: H. Okabe, K. Inoue, and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **24**, 108 (1976).
2) Location: *Nanakuma, Nishi-ku, Fukuoka.*
3) K.D. Roberts, Ek. Weiss, and T. Reichstein, *Helv. Chim. Acta*, **46**, 2886 (1963); **49**, 316 (1966); **50**, 1645 (1967).

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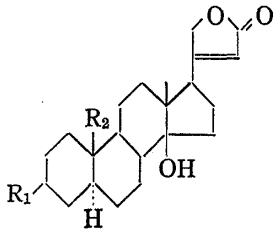
eluate from the polyamide column using 30% MeOH–$CHCl_3$ as a developing solvent caused conversion of a part of I into a more polar Kedde positive compound. Conversion of I was proved to be fairly facilitated by exposure to the sun-light in the non-aqueous solvents, and isolation of I was succeeded by repeated shaded silica gel column chromatography using $CHCl_3$–MeOH–$H_2O$ (7 : 3 : 1, bottom layer) as an eluting solvent. I could not be obtained crystalline, but analysed for $C_{41}H_{62}O_{19}\cdot 2.5H_2O$. Infrared (IR) and ultraviolet (UV) spectra clearly showed it to be a cardenolide glycoside and its nuclear magnetic resonance (NMR) spectra depicted a singlet ($\delta$ 9.52) assignable to the aldehydic proton, signals due to the butenolide protons ($\delta$ 5.61, singlet, $C_{22}$–H; 4.84, 4.61, AB quartet, $J_{AB}$=18 Hz, $C_{21}$–$H_2$), three anomeric protons ($\delta$ 4.60, singlet; 4.33, doublet, $J$=6 Hz; 4.15, doublet, $J$=6 Hz) and one angular methyl group ($\delta$ 0.80, singlet). On acid hydrolysis I gave rhamnose and glucose, and the molecular formula and the NMR spectrum indicated that the sugar moiety is composed of one mole of rhamnose and two moles of glucose. When I was incubated in water with $\beta$-glucosidase at 38° for 2 hours, it gave a glucorhamnoside (II): mp 194—198°, $[\alpha]_D$ −18.5°, UV: 218 nm, $C_{35}H_{52}O_{14}$, NMR: $\delta$ 9.52 (singlet, aldehydic H), 5.61 (singlet, $C_{22}$–H), 4.84, 4.61 (AB quartet, $J_{AB}$=17 Hz, $C_{21}$–$H_2$), 4.62 (singlet, anomeric H of rhamnose), 4.33 (doublet, $J$=6 Hz, anomeric H of glucose). On the other hand, when I was incubated in 30% ethanol for overnight, a rhamnoside (III) was obtained: mp 187—191°, $[\alpha]_D$ −14.1°, UV: 218 nm, $C_{29}H_{42}O_9\cdot 1.5H_2O$, NMR: $\delta$ 9.54 (singlet, aldehydic H), 5.62 (singlet, $C_{22}$–H), 4.84, 4.61 (AB quartet, $J_{AB}$=18 Hz, $C_{21}$–$H_2$), 4.63 (singlet, anomeric H of rhamnose). III and II were identical with previously reported corotoxigenin $\alpha$-L-rhamnopyranoside and $\beta$-D-glucopyranosyl-(1→4)-$\alpha$-L-rhamnopyranoside, respectively in all respects.

I was methylated and methanolysed in the same way as described in the preceding paper. The methanolysate gave on the gas chromatogram the peaks with the same $t_R$ values with those of methyl pyranosides of 2,3,4,6-tetra-O-methyl-$\alpha$-D-glucose, 2,3,4-tri-O-methyl-$\alpha$-D-glucose and 2,3-di-O-methyl-$\alpha$-L-rhamnose, thus indicating that the terminal glucose is linked to the $C_6$-hydroxyl group of the glucose of II. The molecular rotation difference (−94°) between I and II, and the coupling constant (6 Hz) of the anomeric proton indicated that the terminal glucose is $\beta$-D-glucopyranose.




I : $R_1$=$\beta$-D-Glu. pyr.-(1→6)-$\beta$-D-Glu. pyr.-(1→4)-$\alpha$-L-Rham. pyr.-, $R_2$=-CHO
II : $R_1$=$\beta$-D-Glu. pyr.-(1→4)-$\alpha$-L-Rham. pyr.-, $R_2$=-CHO
III : $R_1$=$\alpha$-L-Rham. pyr.-, $R_2$=-CHO
IV : $R_1$=-OH, $R_2$=-COOH
V : $R_1$=-$OCOCH_3$, $R_2$=-COOH

Therefore I was determined to be corotoxigenin $\beta$-D-glucopyranosyl-(1→6)-$\beta$-D-glucopyranosyl-(1→4)-$\alpha$-L-rhamnopyranoside.

The polar compound which was obtained in the course of silica gel chromatography or by exposure of the $CHCl_3$–MeOH solution of I to the sunlight gave an aglycone (IV) on hydrolysis with the crude hesperidinase. It was crystallized from benzene–MeOH to give colorless prisms: $C_{23}H_{32}O_6$, mp 245—256° (decomp.). It was identified with uzarigenin 19-acid by comparison of physical properties of the aglycone and its acetate (V) with those reported.[4]

## Experimental[5]

**Isolation of I**——The intermediate emulsion layer formed on butanol extraction of the aqueous layer[1] was separated and the solvent was evaporated. The residue (3.08 g) was dissolved in water and passed

4) A. Hunger and T. Reichstein, *Helv. Chim. Acta*, **35**, 1073 (1952).
5) For general methods and items employed in this experiment, refer to the experimental of the preceding report.

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through a column (21 cm × 2.5 cm$\phi$) of a 1: 1 mixture of polyamide and celite 545. The eluate by water was evaporated *in vacuo* to a colorless powder (2.5 g). It was repeatedly chromatographed over silica gel packed in a glass tube covered with a sheet of aluminum foil. By eluting with $CHCl_3$–MeOH–$H_2O$ (7: 3: 1, bottom layer), a thin-layer chromatographically homogeneous syrup was obtained and it was puffed and dried *in vacuo* to an amorphous powder (I: 594 mg): $[\alpha]_D^{24}$ −25.8° ($c$=1.50, MeOH), $[M]_D$ −221.4°, IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3500—3000, 2910, 2850, 1750—1700, 1640, 1200—950. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 218 (4.20). NMR[6]: $\delta$ 9.52 (1H, s, -CHO), 5.61 (1H, s, $C_{22}$–H), 4.84, 4.61 (1H each AB quartet, $J_{AB}$=18 Hz, $C_{21}$–$H_2$), 4.60 (1H, s, anomeric H of L-rhamnose), 4.33 (1H, d, $J$=6 Hz, anomeric H of D-glucose), 4.15 (1H, d, $J$=6 Hz, anomeric H of D-glucose), 0.80 (3H, s, angular methyl). *Anal.* Calcd. for $C_{41}H_{62}O_{19}\cdot 2.5H_2O$: C, 54.36; H, 7.40. Found: C, 54.38; H, 7.29.

**Enzymic Hydrolysis of I**——(1) Isolation of II: I (280 mg) and $\beta$-glucosidase (430 mg) were dissolved in water (8 ml), and the mixture was shaken for 2 hr at 38°. EtOH (20 ml) was added and evaporated off to dryness *in vacuo*. The residue was chromatographed over neutral alumina (grade III, 20 g) using $CHCl_3$–MeOH–$H_2O$ (7: 3: 1, bottom layer). The thin-layer chromatographically homogeneous fractions were combined and evaporated. The residue was crystallized from aqueous MeOH to give colorless plates (II: 142 mg): mp 194—198°, $[\alpha]_D^{24}$ −18.5° ($c$=1.00, MeOH), $[M]_D$ −128°, UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 218 (4.18). IR $\nu_{max}^{KBr}$ $cm^{-1}$: superimposable on the spectrum of corotoxigenin $\beta$-D-glucopyranosyl-(1→4)-$\alpha$-L-rhamnopyranoside. NMR: $\delta$: 9.52 (1H, s, -CHO), 5.61 (1H, s, $C_{22}$–H), 4.84, 4.61 (1H each, AB quartet, $J_{AB}$=17 Hz, $C_{21}$–$H_2$), 4.62 (1H, s, anomeric H of L-rhamnose), 4.33 (1H, d, $J$=6 Hz, anomeric H of D-glucose), 1.27 (doublet mounting over other methylene signals, $J$=4 Hz, methyl group of L-rhamnose), 0.79 (3H, s, angular methyl). *Anal.* Calcd. for $C_{35}H_{52}O_{14}$: C, 60.34; H, 7.47. Found: C, 60.17; H, 7.39.

(2) Isolation of III: I (169 mg) and $\beta$-glucosidase (300 mg) were dissolved in 30% aqueous EtOH (10 ml) and the mixture was shaken at 38° overnight. EtOH (20 ml) was added and evaporated to dryness. The residue was chromatographed over neutral alumina (grade III, 20 g). The eluates by $CHCl_3$–MeOH–$H_2O$ (7: 2: 1, bottom layer) showing a single spot were combined and crystallized from aqueous MeOH to give fine needles (III: 25 mg): mp 187−191°, $[\alpha]_D^{27}$ −14.1° ($c$=1.00, MeOH), UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 218 (4.20). IR $\nu_{max}^{KBr}$ $cm^{-1}$: superimposable on the spectrum of corotoxigenin $\alpha$-L-rhamnopyranoside. NMR: $\delta$ 9.54 (1H, s, -CHO), 5.62 (1H, s, $C_{22}$–H), 4.84, 4.61 (1H each, AB quartet, $J_{AB}$=18 Hz, $C_{21}$–$H_2$), 4.63 (1H, s, anomeric H of L-rhamnose), 0.80 (3H, s, angular methyl). The signal of the methyl group of L-rhamnose could not be clearly discernible because of overlapping on the other signals. *Anal.* Calcd. for $C_{29}H_{42}O_9\cdot 1.5H_2O$: C, 62.03; H, 8.02. Found: C, 62.40; H, 8.00.

**Identification of Sugars**——A glycoside (20 mg) was suspended in 1N aqueous HCl (1 ml) and heated in a boiling water bath for 1 hr. The reaction mixture was worked up as usual manner and subjected to paper chromatography (developing solvent: BuOH–AcOH–$H_2O$ (4: 1: 5, top layer)). I and II gave two spots (*Rf*: 0.19, 0.39) while III gave one spot (*Rf*: 0.39). (L-rhamnose: 0.39; D-glucose: 0.19).

**Methylation of I and Identification of Component Methylated Sugars**——Methylation and methanolysis were performed in the same way as described in the preceding paper. The methanolysate was checked by TLC [hexane–AcOEt (1: 2)] and GLC (5% 1,4-butanediol succinate on Shimalite W, 1.7 m × 3 mm $\phi$, 171°, carrier $N_2$, 0.8 kg/$cm^2$): methanolysate: *Rf* 0.47, 0.66, 0.32; $t_R$: 3.5, 8.2. methyl 2,3-di-O-methyl-$\alpha$-L-rhamnopyranoside: *Rf* 0.47; $t_R$ 3.5. methyl 2,3,4,6-tetra-O-methyl-$\alpha$-D-glucopyranoside: *Rf* 0.66; $t_R$ 3.5. methyl 2,3,4-tri-O-methyl-$\alpha$-D- glucopyranoside: *Rf* 0.32; $t_R$ 8.2.

**Autoxidation of I and Isolation of Its Aglycone (IV)**——I (200 mg) was dissolved in MeOH–$CHCl_3$ (2: 1) and allowed to stand in the sunny place for 3 days and the chemical change was monitored by TLC. During this period a polar spot grew in intensity while the amount of I decreased. The solvent was evaporated and the residue was dissolved in water (2 ml) and incubated overnight with the crude hesperidinase (120 mg) at 38°. After evaporation of water the residue was chromatographed over silica gel (10 g). Elution by 10% MeOH in $CHCl_3$ furnished a crystalline powder, which was crystallized from benzene–MeOH to give colorless prisms (IV: 62 mg): mp 245—256° (decomp.), $[\alpha]_D^{25}$ +43.3° ($c$=2.25, MeOH), IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3540, 3100, 2960, 2930, 2870, 1818, 1730, 1705, 1618. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 218 (4.31). NMR: $\delta$ 5.60 (1H, s, $C_{22}$–H), 4.84, 4.59 (1H each, AB quartet, $J_{AB}$=18 Hz, $C_{21}$–$H_2$), 0.80 (3H, s, angular methyl). *Anal.* Calcd. for $C_{23}H_{32}O_6$: C, 68.32; H, 7.92. Found: C, 67.79; H, 7.98. Acetate (V) ($Ac_2O$-pyridine): colorless prisms from benzene–MeOH, mp 226—229° (decomp.) $[\alpha]_D^{25}$ +30.9° ($c$=0.9, MeOH), UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 220 (4.36). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3450, 2975—2880, 1790, 1752, 1725, 1630. NMR: $\delta$ 5.58 (1H, s, $C_{22}$–H), 4.89, 4.66 (1H each, AB quartet, $J_{AB}$=18 Hz, $C_{21}$–$H_2$), 1.95 (3H, s, $-OCOCH_3$), 0.84 (3H, s, angular methyl). *Anal.* Calcd. for $C_{25}H_{34}O_7\cdot 1/2H_2O$: C, 65.93; H, 7.63. Found: C, 66.30; H, 7.56.

6) All NMR spectra were obtained at 100 MHz in the $CDCl_3$–$CD_3OD$ (1: 1) solution.

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