SYNTHESIS AND PHARMACOLOGY OF MANGOSTIN-3,6-DI-*O*-GLUCOSIDE

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Abstract.—A glucoside of mangostin was synthesized and its structure has been determined as mangostin-3,6-di-*O*-glucoside on the basis of spectral and analytical data. A study of its pharmacology indicated it to have a cns depressant activity. The diglucoside also produced a significant rise in blood pressure.

The rind of the fruit of *Garcinia mangostana* Linn. (Guttiferae) has been known to be astringent and has been used in the treatment of diarrhea and dysentry (1). Schmid was the first to isolate mangostin from the fruit hulls of this plant (2). The classical work on the structural elucidation of mangostin by Yates *et al.* (3) established that mangostin is the xanthone represented by structure **1**.

The recent review article by Hostetmann and Wagner (4) on the xanthone glycosides prompted us to prepare the glycoside of mangostin to study its pharmacological activity. Earlier, in connection with our study on the minor constituents of *G. mangostana* (5), we isolated a good quantity of mangostin. This paper reports the details of the synthesis of mangostin-3,6-di-*O*-glucoside and records some interesting pharmacological activity of this diglucoside.

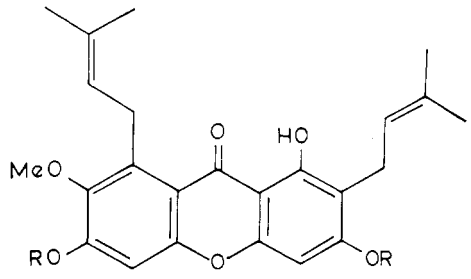
DISCUSSION

Mangostin was isolated from the rind of the fruit of *G. mangostana* (collected in Madras, India) by initial hexane extraction followed by chromatographic separation on a silica gel column. The identity of this xanthone was confirmed by its spectral (uv, ir, nmr and mass) and elemental analytical data. The α-acetobromoglucose required to form the glycosidic linkage was prepared from the readily available β-D-glucose penta-acetate by a modification of Fischer's procedure (6). The diglucoside was prepared according to a procedure reported by Nagarajan and Parmar (7).

A mixture of mangostin, anhydrous calcium sulphate, and freshly prepared silver nitrate was shaken with α-acetobromoglucose (prepared just prior to use) in dry pyridine for 4 hr. After work-up a yellow crystalline solid was obtained. Its spectral data indicated this compound to be a di-(tetra-*O*-acetyl)-glucoside of mangostin represented by structure **2**. Elemental analysis was satisfactory for the molecular formula $C_{52}H_{62}O_{24}$. The uv spectrum of this derivative was not significantly different from that of mangostin. The ir spectrum indicated the presence of an acetyl carbonyl function. The nmr spectrum was consistent with structure **2** for the diglucoside octa-acetate. Especially, the occurrence of a very low field proton at δ 13.33 denoted a chelated hydroxyl group present at position 1 of the xanthone nucleus thus indicating that the two glycosidic linkages are

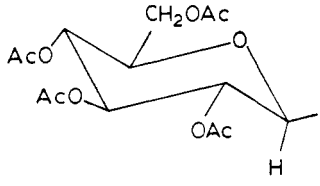
formed with the phenolic hydroxyl groups present at positions 3 and 6. The mass spectrum of **2**, as expected, showed only the molecular ion peak due to the aglycone part (M^+ for $C_{24}H_{26}O_6$ at *m/e* 410) and its further fragmentations. The hydrolysis of the diglucoside octa-acetate was effected by shaking a solution of it with sodium methoxide in methanol followed by neutralization with methanolic acetic acid. The product thus obtained gave satisfactory spectral and analytical data confirming its structure as mangostin-3,6-di-*O*-glucoside (**3**).

1.



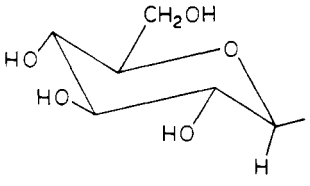
1 R=H Mangostin

2. R =



Di-(tetra-*O*-acetyl)glucoside of Mangostin

3. R =



Mangostin-3,6-di-*O*-glucoside

EXPERIMENTAL

PREPARATION OF α-ACETOBROMOGLUCOSE.—β-D-Glucose penta-acetate (10 g) was dissolved in freshly distilled glacial acetic acid, and the solution was cooled at 15°. Bromine (25 ml) was added dropwise to tetraline (70 ml), and the liberated HBr was passed into the acetic acid solution with care being taken to maintain the temperature between 15–20°. After 2 hr the reaction mixture was poured into ice and water (1 liter) and extracted immediately with chloroform (3 x 50 ml). The chloroform extract was rapidly washed with cold water (2 x 50 ml), dried over anhydrous calcium chloride and filtered. The filtrate was concentrated *in vacuo* at 45° to about 20 ml. Dry petroleum-ether (bp 40–60) was added and the solution was cooled to about 10°. The colorless crystalline needles of α-acetobromoglucose, mp 86° (lit. (6) mp 87–88°) were filtered, dried, and used immediately.

PREPARATION OF DI-(TETRA-O-ACETYL)GLUCOSIDE OF MANGOSTIN (**2**).—Mangostin (2.5 g) was mixed with anhydrous $CaSO_4$ (2.5 g) and freshly prepared Ag_2CO_3 (from $AgNO_3$ (7 g) and excess K_2CO_3 solution) and the suspension was shaken in dry pyridine (50 ml) for 1 hr. α-Acetobromoglucose (10 g) was added, and the reaction mixture was stirred for 3 hr. It was then filtered and the filtrate was added to acetic acid (20%; aqueous; 500 ml). The precipitated solid was filtered and washed with dilute acetic acid. After drying, the solid product was boiled with methanol (200 ml) and filtered again. The filtrate was concentrated and left overnight. The yellow solid (1.5 g) which deposited was recrystallized from methanol as pale yellow crystals, mp 223–225°; uv λ max (EtOH) 240, 261, 304 and 355 nm (log ε 4.45, 4.52, 4.32, and 3.78); nmr ($CDCl_3$) δ 1.69 and 1.82 (broad signals, 12 H, two gem-dimethyl groups), 2.08 and 2.13 (2s, 24H, eight $OCOCH_3$ groups), 3.32 and 4.15 (multiplets, 4H, benzylic methylenes at C_2 and C_5 of the xanthone nucleus), 3.76 (s, 3H, OCH_3), 5.32 (broad singlet, 2H, two $-CH=C>$ groups), 6.46 (s, 1H, C_4–H), 6.92 (s, 1H, C_5–H) and 13.33 (broad singlet, 1H, C_1–OH); ms *m/e* 410 (M^+ for $C_{24}H_{26}O_6$), 395, 393, 381, 367, 354, 163, 43.Anal. calcd for $C_{52}H_{62}O_{24}$; C, 58.30; H, 5.84. Found C, 58.22; H, 5.88.

PREPARATION OF MANGOSTIN-3,6-DI-O-GLUCOSIDE (3).—The di-(tetra-*O*-acetyl)glucoside of mangostin (500 mg) was suspended in dry methanol (50 ml), and a solution of NaOMe (500 mg) in methanol (20 ml) was added. The mixture was stirred until a clear solution was obtained. After 2 hr at room temperature, the solution was neutralized with methanol-acetic acid (1:1 mixture). The solution was then concentrated *in vacuo* at 40°. The solid residue was washed with water and dried. It was then recrystallized from methanol as a yellow solid (360 mg). The mangostin-3,6-di-*O*-glucoside thus obtained had a mp of 245° dec; nmr ($CDCl_3$+CD_3SOCD_3) δ 1.64 and 1.78 (broad singlets, 4H, two benzylic methylenes at C_2 and C_8 of the xanthone nucleus), the protons of the glucose units appeared as broad complex signals in the region 3.30–5.30 and 6.61 (s, 1H, C_4–H), 7.12 (s, 1H, C_5–H) and 13.45 (s, 1H, C_1–OH); ms *m/e* 410 (M^- for $C_{24}H_{26}O_6$), 381, 367, 354 and 163. Anal. calcd. for $C_{34}H_{46}O_{16}$: C, 57.45; H, 6.52. Found: C, 57.68; H, 6.36.

PHARMACOLOGICAL STUDIES

MATERIALS AND METHODS

ANIMALS: Swiss albino mice (20–30 g) and Wistar albino rats (100–150 g) of either sex were used. They were fed and housed for a week in the laboratory animal house prior to the experiment. For cardiovascular studies, adult healthy mongrel dogs (8–12 kg) were used. They were procured from the Corporation of Madras and kept in the laboratory animal house for at least two days prior to experimentation.

DRUG PREPARATION AND ADMINISTRATION: Since mangostin-3,6-di-*O*-glucoside was not freely soluble in distilled water, a fine suspension of the glucoside was prepared with 2% gum acacia (w/w) in a 'Remi homogenizer' at a speed of 3500 rpm. The drug was injected intraperitoneally into rats and mice, and the volume of the suspension was kept at 5 ml/kg. The same volume of 2% gum acacia suspension was injected intraperitoneally into the control animals.

GROSS BEHAVIOR: The effect of mangostin-3,6-di-*O*-glucoside on the gross behavioral pattern of the animals was studied according to the method of Turner (8). The drug was administered to groups of 5 mice each in doses of 50, 100, 200 and 300 mg/kg i.p. Gross behavioral changes were recorded at 15, 30, 60 and 120 minutes after drug administration and were compared with the vehicle-treated control group. To eliminate the subjective errors of the experimenter, often encountered while assessing the spontaneous motor activity of the animals, a modified S.M.A. counter (9) was used, and the spontaneous motor activity of the animal was recorded on a smoked drum before and after the administration of the drug. The effect of mangostin-3,6-di-*O*-glucoside on the spontaneous motor activity of mice was also compared with standard CNS depressants and CNS stimulants such as chloropromazine (5 mg/kg i.p. and d-amphetamine (5 mg/kg i.p.) and recorded on a smoked drum.

PENTOBARBITAL SLEEPING TIME: Mangostin-3,6-di-*O*-glucoside or an equivalent volume of 2% gum acacia was injected 30 min before the administration of 30 mg/kg i.p. of pentobarbital (pentabarbitone sodium, Loba Chemie Wien Fischamend) to groups of 10 animals each of albino rats. The duration of sleep was assessed as the time between the loss and return of the sighting reflex. Results are expressed as percent increase in sleeping time of drug pretreated rats versus those of the control groups.

EFFECT ON BLOOD PRESSURE, RESPIRATION AND INTESTINE *in situ* OF ANAESTHETIZED DOG: Mongrel dogs of either sex were anaesthetized with pentobarbitone sodium (30 mg/kg i.v.). Carotid blood pressure, respiration and intestinal movements were recorded according to the procedure described by Jackson (10). Drugs were injected through the cannulated femoral vein. Mangostin-3,6-di-*O*-glucoside or an equivalent volume of vehicle was administered at dose levels of 5, 10 and 25 mg/kg i.v.: their effects on blood pressure, respiration, and intestinal movements were recorded on a smoked kymograph; five dogs were used for the above experiments.

RESULTS AND DISCUSSION

The vehicle (2% gum acacia) did not show any pharmacological activity in the volume used. Mangostin-3,6-di-*O*-glucoside produced definite signs of CNS depression in the gross behavioral studies only at doses of 100 mg/kg i.p. and above. At doses of 100 and 200 mg/kg ptosis, sedation and decreased motor activity were observed in all of the test animals. There was no significant difference in the behavioral pattern of the experimental animals at doses above 300 mg/kg. The behavioral changes reached a peak by 45 min after drug administration, were

maintained up to 90 min and then gradually declined by 150 min. The recording of the spontaneous motor activity of the animals before and after the administration of the drugs clearly indicated the depressant activity of mangostin-3,6-di-*O*-glucoside (see fig. 1).

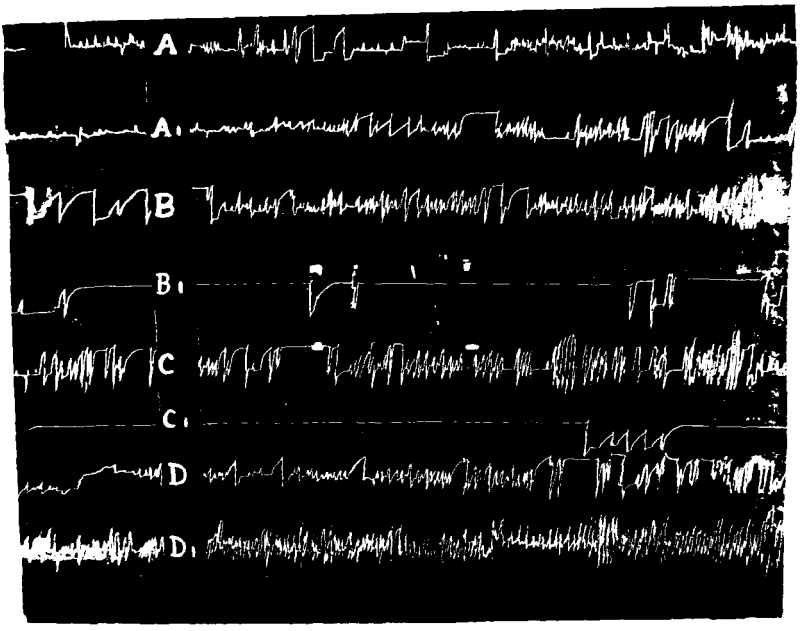


FIG. 1. *Spontaneous Motor Activity of Mice.*

A Control; before administering 2% gum acacia
A_1 Control; after administration of 2% gum acacia
B Mangostin-3,6-di-*O*-glucoside; before administration
B_1 Mangostin-3,6-di-*O*-glucoside; 45 min after administration; 200 mg/kg
C Chlorpromazine; before administration
C_1 Chlorpromazine; 45 min after administration; 5 mg/kg
D d-Amphetamine; before administration.
D_1 d-Amphetamine; 45 min after administration; 5 mg/kg

Mangostin-3,6-di-*O*-glucoside (100 mg/kg i.p.) significantly potentiated the pentobarbital sleeping time;—doses below 100 mg/kg had no significant effect. Rats pretreated with mangostin-3,6-di-*O*-glucoside slept for 119.2 ± 3.11 min (SEM) as compared to 79.3 ± 3.19 min (SEM) sleeping time in the gum acacia treated control groups. The percentage increase in the sleeping time with mangostin-3,6-di-*O*-glucoside treated rats is 53.81.

Intravenous administration of mangostin-3,6-di-*O*-glucoside produced a significant rise in blood pressure, relaxation of intestine and apnea at a dose level of 25 mg/kg. Doses below this level had no significant effect. The rise in blood pressure persisted even after the α-receptors were blocked by intravenous administration of Priscol (5 mg/kg), a dose which completely blocked the pressor effect of intravenously injected adrenaline (5 mg/kg) (fig. 2). Propanalol (0.1 mg/kg) partially blocked the hypertensive effect of mangostin-3,6-di-*O*-glucoside. These preliminary results clearly indicate that further studies are called for before any cardiotonic effects could be assessed.

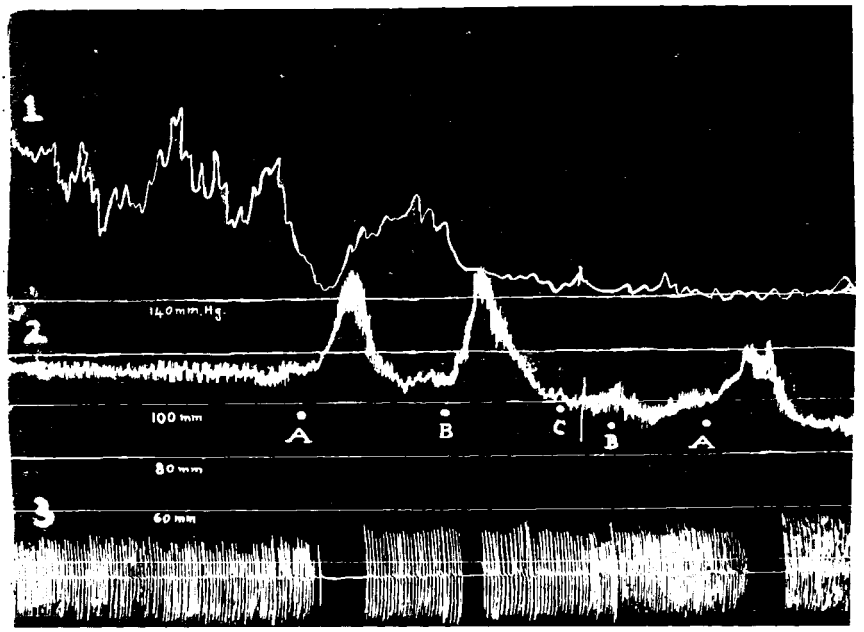


FIG. 2. Effect of Mangostin-3,6-di-O-glucoside on (1) Intestinal movements (2) Blood pressure and (3) Respiration of Anaesthetised dog.

A.	Mangostin-3,6-di-*O*-glucoside	25 mg/kg i.v.
B	Adrenaline	5 mg/kg i.v.
C	Priscol	5 mg/kg i.v.

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