ALKALOIDS OF *Rhinopetalum stenantherum*.

II. THE STRUCTURE OF STENANTHINE AND STENANTHIDINE

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From a methanolic extract of the epigeal part of *Rhinopetalum stenantherum* have been isolated β-chaconine (I) and the new glucoalkaloids stenanthine with mp 262-264°C $[\alpha]_D$ 46.5°, $C_{45}H_{73}NO_{15}$ (II), and stenanthidine with mp 269-271°C, $[\alpha]_D$ −47.5°, $C_{39}H_{63}NO_{11}$ (III). On the basis of the facts that partial hydrolysis of the trioside (II) formed the biosides (I) and (III), and that on the hydrolysis of the latter the monoside γ-chaconine was found, it may be assumed that stenanthine has the structure of solanidine 3-O-{[O-β-D-glucosyl-(1 → 6)]-[O-α-L-rhamnosyl-(1 → 4)]-D-glucoside}, and stenanthidine that of solanidine 3-O-[O-β-D-glucosyl-(1 → 6)-D-glucoside].

Continuing an investigation of the alkaloids of the epigeal part of *Rhinopetalum stenantherum* (family Liliaceae; collected on March 28, 1978 in Kamplanbeke), by chloroform and methanolic extraction we obtained 0.3% of total alkaloids [1]. From the total alkaloids isolated by methanolic extraction, using silica gel column chromatography, we have obtained bases with mp 253-255°C, $[\alpha]_D$ −61.4°, $C_{39}H_{63}NO_{10}$ (I), with mp 262-264°C $[\alpha]_D$ −46.5°, $C_{45}H_{73}NO_{15}$ (II), and with mp 269-271°C $[\alpha]_D$ −47.5°, $C_{39}H_{63}NO_{11}$ (III).

The IR spectra of (I-III) are similar. Each of them shows the absorption bands of hydroxy groups (3420 cm^{-1}), and of a double bond (1640 cm^{-1}) and a broad band of the stretching vibrations of C—OH groups (1000-1150 cm^{-1}) that is characteristic for glycoalkaloids [2, 3]. The mass spectrum of (II) has the peak of the molecular ion with m/z 705 (16) and peaks corresponding to the alkaloid solanidine [4]: 397 (5.3), 396 (5.5), 380 (23), 204 (25), and 150 (100). From its melting point, composition, and specific rotation, base (I) is similar to the alkaloid β-chaconine. In actual fact, the hydrochloric acid hydrolysis of base (I) gave an aglycone with mp 216-218°C and composition $C_{27}H_{43}NO$ (IV) identical with solanidine (mixed melting point, IR spectrum). D-Glucose and L-rhamnose were found in the carbohydrate moiety (GLC and paper chromatography). The partial hydrolysis of (I) gave a compound (IV) and a base with mp 242-244°C $[\alpha]_D$ −38.8°, $C_{33}H_{53}NO_6$ (V), M^+ 559, which from its specific rotation, melting point, IR and mass spectra, and chromatographic behavior in TLC in the presence of a marker was identical with γ-chaconine [5-7]. The facts given above show that the base (I) is β-chaconine [6, 8].

Bases (II) and (III) proved to be new and we have called them stenanthine and stenanthidine, respectively. They are sparingly soluble in acetone, alcohols, and chloroform. The mass spectrum of stenanthine has the peaks of ions with m/z 867, M^+ (0.1), 721 (1.3), 705 (15), 690 (2.2), 671 (5.5), 656 (1.8), 559 (31), 544 (9.0), 397 (11), 396 (10), 380 (31), 204 (16), 150 (100). The mass spectrum of stenanthidine has the peaks of ions with m/z 721, M^+ (4.5), 706 (2.0), 577 (68), 544 (19), 397 (11), 396 (11), 380 (26), 204 (56), 150 (100). The hydrolysis of (II) and (III) formed the same aglycone, which was again identical with solanidine (mixed melting point, IR and mass spectra). The carbohydrate moiety of (II) contains glucose and rhamnose (2:1), and that of (III) 2 molecules of D-glucose (GLC and paper chromatography). The acetylation of (II) gave amorphous acetyl stenanthine in the IR spectrum of which the absorption band of the hydroxy group was absent and the absorption bands of an ester carbonyl (1755, 1230 cm^{-1}) appeared.

The partial hydrolysis of stenanthine gave a mixture of four substances the chromatography of which on a column of silica gel yielded solanidine, γ-chaconine, stenanthidine, and β-chaconine, which were identified by mixed melting points. The partial hydrolysis of

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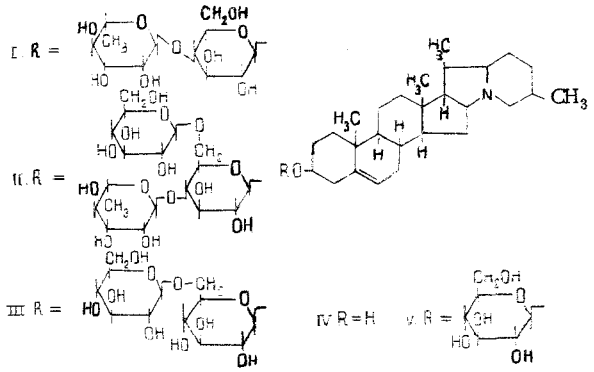
stenanthidine led to the formation of (IV) and (V). The type of linkage between the glucose residues was investigated with the aid of periodate oxidation and methylation, and also by means of the results of a study of the mass spectra of the permethylates of stenanthine (VI), β-chaconine (VII), and of stenanthidine (VIII). The formation of the glycoalkaloids of (I) and (III) by the partial hydrolysis of (II) showed the branched nature of the oligosaccharide moiety of the trioside molecule.

Acid hydrolysis of the Hakomori-methylated [9] stenanthine yielded 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,4-tri-O-methyl-L-rhamnose, and 2,3-di-O-methyl-D-glucose, which were identified with the aid of TLC and GLC in the presence of authentic samples. It follows from this that terminal glucose and rhamnose residues are attached to the fourth and sixth hydroxyls of the glucose directly bound to the solanidine. This type of branching was confirmed by the results of the periodate oxidation of (II), in which all the sugars were destroyed, and also by the absence of a reaction with the Bonner reagent [10, 11] of the sugar methyl esters obtained from the permethylate (II).

The calculation of molecular rotation differences [12] showed the β configuration of the glycosidic bonds. This was confirmed by the oxidation of a hydrolysate of (II) by β-glucose oxidase, the product of which revealed no glucose spots on paper chromatography [17].

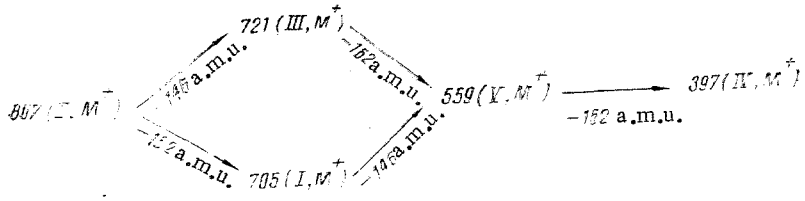
Since, as the result of the partial hydrolysis of the trioside (II), the biosides (I) and (III) were formed, and the hydrolysis of the latter gave the monoside (V), it may be concluded that stenanthine has the structure of solanidine 3-O-{[O-β-D-glucosyl-(1 → 6)][O-α-L-rhamnosyl-(1 → 4)]-D-glucoside} (II) and stenanthidine that of solanidine 3-O-[O-β-D-glycosyl-(1 → 6)-D-glucoside] (III).



In order to confirm the structure of the new bases and to check the structural possibilities of the method for similar substances we investigated the mass spectra of the glycoalkaloids (I-III) and (V) and of the permethylates (VI-VIII).

As stated above, the mass spectra of the trioside (II), the biosides (I) and (III), and the monoside (III) contain the peaks of the molecular ions. The aglycone moieties of all four glycoalkaloids were represented by the peaks of ions with m/z 397 ($C_{27}H_{43}NO$), 380 ($C_{27}H_{42}N$), 204 ($C_{14}H_{22}N$), and 150 ($C_{10}H_{16}N$), which is characteristic for solanidine [4].

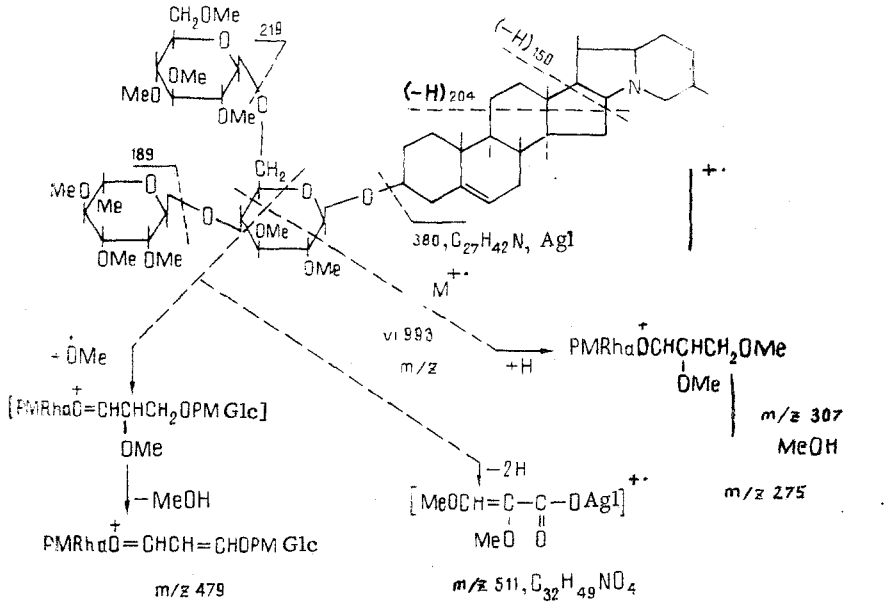
The fragmentation of (II) under electron impact was similar to the pattern of the hydrolytic cleavage of compounds (I-III) and (V):



The spectrum of stenanthine permethylate (VI) has a strong peak of the molecular ions with m/z 993 ($C_{54}H_{91}NO_{15}$). Fragments with m/z 219 and 189, and also products of their decomposition confirm the presence in the trioside molecule of two terminal sugar residues, glucose and rhamnose. This is also shown by the presence of the ions $(M - 162)^+$ and $(M - 146)^+$ in the spectrum of the initial base (II). The spectra of the permethylates of the bases (I) and (III), (VII) and (VIII) contain, correspondingly, the peaks of ions with m/z 189 (VII) and 219 (VIII), which shows the presence of one terminal hexose.

We have turned our attention to the peak of the fragment with m/z 479, composition $C_{22}H_{39}O_{11}$, in the spectrum of the permethylate (VI). The composition of this ion indicates the inclusion of two sugar residues connected by a propylidene chain: PMRha$\overset{+}{O}$=CHCH=CHOGlc-PM*. We have previously observed ions of analogous composition in the spectra of permethylates of spirostanol triosides with two terminal carbohydrate residues connected to a third by 1 → 2 and 1 → 4 bonds. In essence, these ions are monotypical with the ions having m/z 305 that are characteristic for the permethylates of 1 → 2 and 1 → 4 disaccharides [13]. On the basis of the presence of a propylidene chain between the carbohydrate units in the ions with m/z 479 it is possible to suggest only two variants of the attachment of the terminal pyranose rings in the molecule of the glycoalkaloid (II): 1 → 4 and 1 → 2, or 1 → 4 and 1 → 6. The second variant is more likely, since no sugars were detected by paper chromatography in a hydrolysate of the product of periodate oxidation, which shows the presence of a 1 → 6 bond.

The spectrum of the bioside permethylate (VII) has the peak of an ion with m/z 275 of moderate intensity (PMRha$\overset{+}{O}$=CHCH=CHOMe) [14], which is absent from the spectrum of the permethylate (VIII). The peak of an ion with m/z 305 is not observed in the latter, either. These facts indicate that the rhamnose residue of base (I) is attached by a 1 → 4 bond, and the terminal glucose residue of base (III) by a 1 → 6 bond, since the formation of fragments with m/z 305 is uncharacteristic for this type of bond [13, 15]. It follows from this that the alkaloids considered have the structures (I-III, V), and the ion with m/z 479 is formed as shown in the scheme.



The structure of the permethylate (VI) suggests the possibility of the appearance of an ion with m/z 275 or of its precursor with m/z 307 (scheme). The formation of precursor ions of this type has been considered for the case of permethylates of oleanolic acid glycosides by Kochetkov et al. (XVI). In actual fact, the peak of an ion with m/z 307 ($C_{14}H_{27}O_7$) is present in the spectrum of (VI).

An additional fact confirming the structures (II) and (III) is the presence in the spectra of their permethylates, (VI) and (VIII), of the peak of an ion with m/z 511 ($C_{32}H_{42}NO_4$) formed in the decomposition of the same bonds of the pyranose ring closest to the aglycone as for the ion with m/z 479 (scheme).

EXPERIMENTAL

Thin-layer chromatography (TLC) was performed in a fixed layer of KSK silica gel containing 5% of gypsum. The same type (50-250 μm) was used for column chromatography, together

*Here and below, PMRha and PMGlc denote residues of permethylated rhamnose and glucose, respectively.

with the solvent systems: 1) chloroform–methanol [a – (10:2), b – (10:1), c – (10:0.5), d – (10:0.1)] and 2) benzene–acetone (2:1). Carbohydrates were detected by paper chromatography (PC), (type FN-11 paper, GDR) in the butan-1-ol–pyridine–water (6:4:3) system. The time of chromatography was 17 h. The spots were revealed with aniline hydrogen phthalate at 100-110°C for 10 min. The methylated sugars were detected by TLC (silica gel) with o-toluidine salicylate as chromogenic agent. Gas–liquid chromatography (GLC) of the free sugars in the form of the trimethylsilyl ethers of the methyl glycosides and methyl glycosides of the methylated carbohydrates was performed by a known method [3].

IR spectra were obtained on a UR-20 spectrometer (KBr tablets), and mass spectra on a MKh-1310 instrument using a system for the direct introduction of the sample. The ionizing voltage was 50 V, the collector current 40 μA, and the temperature of the evaporator 180-250°C for bases (I-V) and 120-130°C for the permethylates (VI-VIII). The elementary compositions of the ions were measured at R = 15,000, the reference substance being perfluorokerosine, and the relative error of the determination of masses did not exceed $5 \cdot 10^{-6}$.

Isolation of the Total Alkaloids. The epigeal part of *Rhinopetalum stenantherum* plants (9 kg) was wetted with 10% ammonia and extracted with chloroform. Extraction was performed eight times. The concentrated chloroform extract was treated repeatedly with 10% sulfuric acid. The acid solution was shaken with ether and was then made alkaline with ammonia, and the alkaloids were extracted with chloroform (7.76 g). The plant remaining after chloroform extraction was dried and extracted with methanol. The concentrated methanolic extract was treated with 5% sulfuric acid. The acid solution was treated in the same way as in the preceding extract. This gave 19 g of combined alkaloids. The total yield of alkaloids was 27.76 g (0.3% of the weight of the dried plant).

Separation of the Total Alkaloids. The total alkaloids isolated by methanolic extraction (19 g) were dissolved in a mixture of chloroform and methanol (1:1) and chromatographed on a column of silica gel (80 × 3 cm) with elution by chloroform–methanol (10:1) and (10:2). A 700-ml fraction was taken and evaporated to dryness. TLC showed the presence of four spots. Then, continuing elution, a total to twenty 250-ml fractions was collected. The material from combined fractions 1-6 (2.56 g) was rechromatographed on a column of silica gel with elution by benzene–methanol (4:1). On treatment with methanol, the first eluates (80 ml) deposited stenanthidine with the composition $C_{39}H_{63}NO_{11}$, mp 269-271°C (methanol), $[\alpha]_D$ –57.5, (c 1.095; pyridine), M^+ 721, R_f 0.20 in system 1a.

The subsequent eluates yielded β-chaconine with mp 253-255°C (methanol). $[\alpha]_D$ –61.4° (c 0.85, pyridine), M^+ 705, R_f 0.17 in system 1a.

After treatment with methanol, the chloroform–methanol (10:2) eluate yielded stenanthine with mp 262-264°C, $[\alpha]_D$ –46.5° (c 0.86; pyridine), M^+ 867, R_f 0.1 in system 1a. Stenanthine was also obtained from the following eluates (chloroform–methanol (10:1)). Found, %: C 61.87; H 8.60; N 1.67. $C_{45}H_{73}NO_{15}$. Calculated, %: C 62.27; H 8.44; N 1.61, M^+ 867.

Hydrolysis of the Base (I). A solution of 70 mg of (I) in 10 ml of 10% hydrochloric acid containing ethanol (1:1) was heated at 100°C for 3 h. The ethanol was evaporated off in vacuum. The reaction mixture was diluted with water (10 ml), made alkaline with ammonia, and extracted with chloroform. The chloroform residue was chromatographed on a column of silica gel and was eluted with chloroform–methanol (10:2). This gave crystals with mp 216-218°C (ethanol), with the composition $C_{27}H_{43}NO$, R_f 0.87 in system 1a, identical with solanidine.

Mass spectrum: m/z 397, M^+ (31), 396 (24), 382 (17), 380 (1.8), 204 (30), 150 (100).

In the carbohydrate moiety, D-glucose and L-rhamnose were detected by GLC and PC.

Partial Hydrolysis of Base (I). A solution of 300 mg of (I) in 20 ml of 5% oxalic acid was boiled for 2 h. The hydrolysate was made alkaline with ammonia and extracted with chloroform. On TLC (system 1a) the chloroform residue showed three spots, with R_f 0.87, 0.51, and 0.17. The dry residue was dissolved in a mixture of chloroform and methanol (10:2) and was chromatographed on a column. Elution was carried out with chloroform–methanol (10:2), 10- to 15-ml fractions being collected. After the thin-layer chromatography of the fractions obtained, similar ones were combined, and three fractions were obtained, the third of which was the initial material. Treatment of the first fraction with ethanol gave solanidine, R_f 0.87. The second fraction yielded crystals with mp 242-244°C (methanol) composition $C_{33}H_{53}NO_6$, $[\alpha]_D$ –38.8° (c 0.67; pyridine), identified as γ-chaconine (V) (mixed melting point).

IR spectrum of (V), cm^{-1}: 3420 (OH), 2955-2860, 1463, 1442 (—CH_3; —CH_2—) 3055, 1645 (C=C), and a broad absorption band at 1025-1110 (C—OH).

Mass spectrum of (V): m/z 559, M^+ (26), 544 (8.3), 397 (5.3), 396 (5.4), 380 (16), 204 (28), 150 (100).

Hydrolysis of Stenanthine. Stenanthine (300 mg) was hydrolyzed in a similar manner to the hydrolysis of base (I). After the appropriate working up, solanidine (IV) was obtained.

D-Glucose and L-rhamnose were detected by TC in the alkaline solution after the removal of the solanidine. GLC showed the presence of glycose and rhamnose in stenanthine (II) in a ratio of 1.00:0.39. After the determination of the sugars by PC, the residue was dissolved in 3 ml of 0.2 M sodium acetate buffer and the enzyme β-glucose oxidase (3 mg) was added. The mixture was incubated at 37°C. After treatment with KU-2 (H^+), it was evaporated in a rotary evaporator. Paper chromatography did not show the presence of residual D-glucose.

Acetylation of Stenanthine. Stenanthine (200 mg) was acetylated with acetic anhydride (3 ml) in pyridine (2 ml). This gave amorphous acetylstenanthine with R_f 0.78 (system 1b).

Partial Hydrolysis of Stenanthine (II) and Stenanthidine (III). In a similar manner to the hydrolysis of base (I), 240 mg of (II) and 50 mg of (III) were hydrolyzed separately with 5% oxalic acid. The resulting mixture of alkaloids isolated from the hydrolysate (II) was chromatographed on a column of silica gel with elution by chloroform—methanol (10:2). This gave solanidine, γ-chaconine, stenanthidine, and β-chaconine, while the hydrolysate of (III) gave solanidine and γ-chaconine.

Stenanthine Permethylate. With stirring, 0.5 g of sodium hydride was added to 150 mg of stenanthine in 15 ml of dimethyl sulfoxide (90 min at room temperature), and then the mixture was shaken for 1 hour. After this, 20 ml of methyl iodide was added over an hour and stirring was continued for another two hours. The reaction product was left for 12 h and was then poured into an aqueous solution of sodium thiosulfate and was extracted with chloroform. On TLC, the permethylate obtained showed a mixture of four substances. The mixture of products was dissolved in dimethyl sulfoxide and remethylated. After the appropriate working up, the permethylate obtained showed two spots. Separation on the column of alumina and elution with chloroform yielded amorphous stenanthine permethylate (VI) with R_f 0.4 (system 1c). The IR spectrum of (VI) lacked absorption in the region of stretching vibrations of OH groups.

Mass spectrum: m/z 993, M^+ (20), 978 (3.2), 859 (0.5), 833 (3.1), 819 (1.6), 804 (0.5), 788 (1.2), 775 (1.0), 758 (0.6), 569 (1.0), 511 (1.1), 479 (2.0), 467 (0.6), 396 (3.5), 380 (58), 307 (3.0), 275 (0.8), 219 (8.0), 204 (22), 189 (55), 187 (51), 157 (15), 150 (100), 101 (42), 88 (57).

Hydrolysis of Stenanthine Permethylate (VI). A solution of 20 mg of (VI) in 4 ml of 10% sulfuric acid was boiled for 6 h. The acid solution was neutralized with barium carbonate and the solid matter was filtered off. The filtrate was evaporated in vacuum. On TLC, the residue showed three spots. With R_f 0.61, 0.48, and 0.27 (system 2). These TLC R_f values were identical with those of authentic 2,3,4-tri-O-methyl-L-rhamnose, 2,3,4,6-tetra-O-methylglucose and 2,3-di-O-methylglucose.

Periodate Oxidation of Stenanthine. A. Stenanthine (40 mg) was treated with 3 ml of 0.3 M $NaHCO_4$ and two liters of 1 M $NaHCO_3$ and the mixture was kept in the dark at 5-6°C for a week. The course of the reaction was checked in TLC in system 1a. A few drops of ethylene glycol were added and over an hour the solvent was evaporated off in vacuum to dryness. The residue was treated with 7 ml of 5% sulfuric acid, the mixture was heated in the water bath for 5 h and was then made alkaline with ammonia and the alkaloids were extracted with chloroform. No sugars were detected by PC in the alkaline solution after its evaporation to the state of a syrup.

B. Stenanthine (60 mg) was oxidized with 25 ml of 0.04 M sodium metaperiodate [10] at 5°C in the dark (15 days). In part of the reaction mixture the consumption of periodate was determined by titration of an aliquot with 0.01 N NaOH. For one molecule of the glucoalkaloid 4.34 moles of $NaIO_4$ were consumed and 1.75 moles of HCOOH liberated. The remainder of the reaction mixture, after the destruction of the excess of periodate with ethylate glycol and evaporation to dryness, was hydrolyzed with 5% sulfuric acid at 100°C for 5 h. No residual sugars were detected in the hydrolysate.

Hydrolysis of Stenanthidine (III). A solution of 60 mg of (III) in 5 ml of 5% sulfuric acid was boiled for 5 h. The hydrolysate was made alkaline with ammonia and extracted with chloroform. The treatment of the chloroform residue with ethanol eluted solanidine.

D-Glucose was detected by PC in the alkaline solution after the elimination of the solanidine.

β-Chaconine Permethylate (VII). Over 60 min, 250 mg of sodium hydride was added to 80 mg of the alkaloid β-chaconine in 8 ml of dimethyl sulfoxide. Then 10 ml of methyl iodide was added and methylation was carried out in a similar manner to the methylation of (II). After working up and separation on a column, the amorphous permethylate (VII) was obtained with R_f 0.85 in system 1d.

Mass spectrum: m/z 789, M^+ (20), 774 (5.0), 657 (0.5), 644 (0.3), 629 (3.0), 614 (1.5), 600 (1.0), 584 (0.4), 396 (2.7), 380 (33), 275 (1.3), 204 (24), 189 (43), 157 (11), 150 (100), 101 (38), 88 (52).

Stenanthidine Permethylate (VIII). In a similar manner to the treatment of (II), 50 mg of stenanthidine in 5 ml of dimethyl sulfoxide was methylated with 200 mg of sodium hydride and 7 ml of methyl iodide. This gave the amorphous permethylate (VIII) with R_f 0.82 in system 1b.

Mass spectrum: m/z 819 M^+ (22), 804 (3.4), 685 (1.2), 644 (1.1), 629 (2.6), 615 (21), 600 (9.2), 555 (8.2), 511 (14), 467 (12), 396 (4.8), 380 (43), 219 (11), 204 (30), 187 (27), 150 (100), 101 (44), 88 (41).

SUMMARY

β-Chaconine and the new glucoalkaloids stenanthine and stenanthidine have been isolated from a methanolic extract of the epigeal part of *Rhinopetalum stenantherum*; stenanthine is solanidine 3-O-{[O-β-D-glucosyl(1 → 6)][O-α-L-rhamnosyl(1 → 4)]-D-glucoside} (II), and stenanthidine is solanidine 3-O-[O-β-D-glucosyl-(1 → 6)-D-glucoside] (III).

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