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The Structure of Mollugocin A. A New Triterpene Glycoside from *Mollugo hirta*

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Mollugocin A—a new triterpene glycoside isolated from *Mollugo hirta* has been shown to be Mollugogenol A-3-[α-L-arabinofuranosyl (1→5)]-[α-L-arabinofuranosyl (1→4)[-β-D-glucopyranoside].

IN a preliminary communication[1] the structure of mollugocin A, a new triterpene glycoside from *Mollugo hirta* (syn. *M. lotoides*), has been proposed as (I). The present paper details the experiments leading to the structure.

Mollugocin A, $C_{46}H_{78}O_{17}$, m.p. 276°–80° (dec.) though obtained in a crystalline state, was found to be hygroscopic in nature. It gave copious lather when shaken with water and haemolysed red blood cells, which are characteristics of saponins. It was also found to be a fish poison. Mollugocin A was obtained in a crystalline state from the ethanolic extract of the defatted plant material. It was purified by repeated crystallization from ethanol.

Mollugocin A on hydrolysis with ethanolic hydrochloric acid yielded mainly mollugogenol A (IIa)[2,3] besides traces of 22-dehydromollugogenol A[2,3] and mollugogenol B[4]. That the latter two products were artefacts formed during acid hydrolysis of mollugocin A was proved by subjecting mollugogenol A to acid hydrolysis under identical condition. Further mollugocin A did not show any triple UV absorption maxima at 243, 251 and 261 nm like mollugogenol B[4]. Moreover enzymatic hydrolysis of mollugocin A with β-glucosidase gave only mollugogenol A indicating mollugocin A to be a glycoside of mollugogenol A.

Mollugocin A on acid hydrolysis with ethanolic sulphuric acid under controlled condition gave L-arabinose and after complete hydrolysis gave D-glucose and L-arabinose. The relative molar ratio of D-glucose and L-arabinose in mollugocin A was found to be 1 : 2 by GLC.

Mollugocin A was first methylated with CH_3I and Ag_2O and then by prolonged Hakomori's method[5]. The methylated aglycone (IIb), $C_{32}H_{56}O_4$, m.p. 201–3°, obtained after acid hydrolysis of the permethylated product did not show the molecular ion peak in the mass spectrum, but showed peaks at m/e 486 and m/e 446 which are due to $(M-H_2O)^+$ and $(M-58)^+$ ions respectively. The loss of 58 mass units from the molecular ion clearly indicated that the tertiary hydroxyl group in the methylated aglycone was free. The peak at m/e 59 was due to ion $(CH_3)_2\ C = \overset{+}{O}H$ formed by the cleavage of the hydroxy isopropyl group. The mass spectrum of the methylated aglycone also showed peaks at m/e 237 which may arise from the part containing A and B ring (ion *a*) or that containing D and E (ion *b*) by the splitting of ring C. There was no peak at m/e 223 as has been observed in the mass spectrum of mollugogenol A.[2,3]

The methylated aglycone was subjected to Jones oxidation which yielded a product (IIC, not isolated in a pure state due to the very poor yield—the oxidation being carried out on a micro scale) which gave positive Zimmerman's colour test for a 3-keto group[6] and this clearly showed that the C-3 hydroxyl group was free in the methylated aglycone. From the above data it was concluded that the saccharide side chain in the mollugocin A is linked through the C-3 hydroxyl group.

After acid hydrolysis of the permethylated product, the sugar components were identified as 2,3,6-tri-*o*-methyl D-glucose, 2,3,5-tri-*o*-methyl L-arabinose and 2,3-di-*o*-methyl L-arabinose respectively.

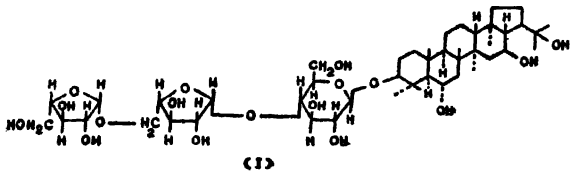
Mollugocin A on hydrolysis with 8% sulphuric acid in methanol for 10 hr, in a sealed tube, and on working up by the usual method[7] was found to contain aglycone 51.22% and sugars 45.02%.

When hydrolysed with β-glucosidase, mollugocin A gave only mollugogenol A and a saccharide not identical with either glucose or arabinose. This indicated that the saccharide chain is linked to mollugogenol A through the glucose moiety. It also confirmed the β-linkage of D-glucose with the aglycone.

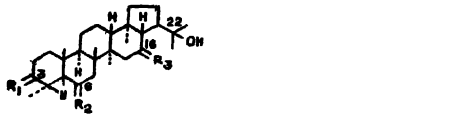
It has been shown earlier that in mollugocin A the saccharide chain is linked to mollugogenol A through the C-3 hydroxyl group. Triterpenoid glycosides are usually C-3-O-glycosides. Identification of the methylated sugars not only suggested the

pyranose ring form for D-glucose and furanose ring form for L-arabinose respectively, but also the straight chain nature of the saccharide unit. The unit which yielded trimethyl arabinose definitely possessed furnose structure but that yielding dimethyl arabinose can be assigned either pyranose or furanose structures. The ease of acid hydrolysis strongly suggests[8] furanose ring form and α-L-glycosidic linkage between the two arabinose units and between arabinose and glucose units. With regard to the configuration of saponin glycosidic linkages it is a general observation that D-sugars occur with β-glycosidic and L-sugars with α-glycosidic linkage[9].

On the basis of the data discussed so far, the structure of mollugocin A can be represented as : Mollugogenol A-3-[α-L-arabinofuranosyl $(1 \rightarrow 5)$]-[α-L-arabinofuranosyl $(1 \rightarrow 4)$]-β-D-glucopyranoside (I).



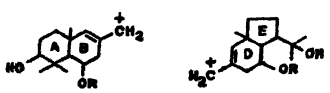
(I)



(II) a, $R_1 = R_3 =$  ; $R_2 =$

b, $R_1 =$  ; $R_2 =$  ; $R_3 =$ 

c, $R_1 = O$; $R_2 =$; $R_3 =$



g, R = CH_3 h, R = CH_3

i, R = H j, R = H

Experimental

Isolation of mollugocin A

Air-dried powdered leaves (1 kg) of *M. hirta* were first defatted with pet. ether (b.p. 60°–80°) and then extracted with ethanol (95%) in a Soxhlet apparatus for 36 hr. The ethanolic extract was then concentrated to a small volume by distillation and kept overnight at the room temp. when a colourless crystalline product separated out, which was filtered and washed repeatedly with ethyl ether. The product was then repeatedly crystallised (20 times) from ethanol (95%); yield (237 mg). It showed single spot in tlc [solvent system (1) *n*-butanol : acetic acid : water = 5 : 1 : 4, v/v and (2) ethanol : acetic acid : water = 5 : 1 : 4, v/v], m.p. 276°–80° (dec.) [m.p. found in a sealed tube], $[\alpha]_D^{20}$ −12.2° (Py).

Acid hydrolysis of mollugocin A—characterization of the aglycons and the sugar constituents

Mollugocin A (200 mg) was hydrolysed by refluxing with ethanolic hydrochloric acid [ethanol 95% (100 ml) and conc. hydrochloric acid (20 ml.)] for 4 hr on a steam bath. The aglycone part was isolated in the usual way. It showed three spots in tlc (solvent system : benzene : chloroform : methanol = 30 : 15 : 5, v/v). The compounds corresponding to the three spots were then separated by preparative tlc using the above solvent system and the three products (all crystallized from aq. methanol) having melting points 250°–52°, 243°–46° and 222°–25° respectively were obtained. These were identified as mollugogenol A, 22-dehydromollugogenol A and mollugogenol B respectively by comparison (m.p., mixed m.p. and IR spectra) with authentic samples.

The aqueous part obtained after filtration of the aglycones was neutralised by Ag_2CO_3, filtered and then evaporated to a syrup. The syrup was subjected to descending paper chromatography [solvent system (i) *n*-butanol : acetic acid : water = 4 : 1 : 1, v/v and (ii) *n*-butanol : ethanol : water = 4 : 1 : 5, v/v; spraying reagent : aniline hydrogen phthalate] when two spots of R_f 0.20 and 0.24 respectively were observed and these corresponded to those of authentic samples of D-glucose and L-arabinose respectively. By preparative paper chromatography D-glucose, (m.p., 146°; $[\alpha]_D$+52.5° (water) and L-arabinose, (m.p. 155°–57°; $[\alpha]_D$+107° (water) were isolated and characterised.

Treatment of mollugogenol A with hydrochloric acid in ethanol

Mollugogenol A (100 mg) was subjected to acid hydrolysis under the above mentioned experimental conditions and three products, mollugegenol A, 22-dehydromollugogenol A and mollugogenol B were obtained.

Determination of relative ratio of D-glucose and L-arabinose in mollugocin A by GLC

The mixture of sugars (10 mg; dried under vac.) obtained by acid hydrolysis of mollugocin A (vide supra) was treated with anhydrous pyridine (1 ml) and hexamethyl disilazane (HMDS, 0.2 ml) and trimethyl chlorosilane (0.1 ml). The reaction was carried out in a glass vial containing teflon lined screw cap. It was shaken vigorously for 1 min. and allowed to stand for 15 min. at room temp. The clear liquid from the top of the reaction mixture was drawn by a Hamilton syringe in a volume and injected directly into the gas chromatograph (F & M Model 700-12R Chromatograph, Hewlett and Packard, U.S.A.). The apparatus was equipped with dual column and dual flame ionisation detector. The columns used were stainless steel tubings (6′ × $\frac{1}{4}$″) packed with 3% SE-30 on Gas Chrom-P (60–80 mesh) obtained from Applied Science Laboratories, Pennsylvania, U.S.A. The conditions for chromatography were as follows : Column oven temperature 140°,

injection Port and detector temperature, 230°. The carrier gas used was nitrogen, with a flow rate of 40 ml per minute at 25 lbs. per sq. inch. The flame was produced by a mixture of hydrogen and compressed air both flowing at the rate of 40 ml per minute at a pressure of 30 lbs. per sq. inch. From the respective area covered by the TMS-D-glucose and TMS-L-arabinose in the vapour phase chromatogram, the relative ratio of D-glucose and L-arabinose in mollugocin A could be calculated as 2.97 : 6.46 = 1 : 2.17, which can be taken as 1 : 2.

Partial hydrolysis of mollugocin A

Mollugocin A (800 mg) was dissolved in 8% sulphuric acid in methanol, (100 ml) and then refluxed on steam bath for 20 min. The mixture was then neutralised by $BaCO_3$, the precipitated $BaSO_4$ and unreacted $BaCO_3$ filtered off, and the solution evaporated to a syrup. The syrup on descending paper chromatography (solvent system : *n*-butanol : acetic acid : water = 4 : 1 : 1, v/v; spraying reagent : aniline hydrogen phthalate) showed only one spot identical with an authentic sample of L-arabinose.

Again the syrup on tlc (solvent system : benzene : chloroform : methanol = 30 : 15 : 5, v/v) showed a spot at the base line, very much polar than those of mollugogenol A, 22-dehydromollugogenol A and mollugogenol B which indicated that partial hydrolysis of mollugocin A had taken place.

Methylation by silver oxide and methyl iodide

Mollugocin A (2 g) was dissolved in CH_3I (150 ml) containing dry methanol (6 ml) and Ag_2O (12 g) was added in several lots over a period of 6 hrs with occasional shaking. The mixture was then refluxed on a steam bath for 16 hr. CH_3I was then distilled off and the residue was extracted with chloroform. The chloroform-extract was dried over anhydrous Na_2SO_4 and the solvent was distilled off. The product obtained was subjected to further methylation for four times by the same process. The final product was again methylated by Hakomori's method.

Permethylation by Hakomori's method

50% NaH dispersion in oil (800 mg) was suspended in DMSO (80 ml) and kept in an oil bath at 80° for 1 hr. A solution of the partially methylated product of mollugocin A (820 mg, vide supra) in DMSO (30 ml) was then added gradually and the mixture was kept at 80° for 3 hr with constant stirring. The mixture was then cooled in ice bath and CH_3I (15 ml) was added to it dropwise. The mixture was kept overnight at the room temp. and then extracted with chloroform and the chloroform-extract was dried over anhydrous Na_2SO_4 and the solvent was distilled off. The syrup obtained was subjected to further methylation for once more by the same process.

Hydrolysis of the above methylated product

The permethylated product (670 mg) was then taken up in methanol (250 ml) and was hydrolysed under reflux for 4 hr after addition of conc. HCl (50 ml). The mixture was poured into a porcelain basin and the methanol was removed on the steam bath keeping the total volume of the mixture constant by gradual addition of water. The sapogenin thus precipitated, was filtered, washed and dried. It was crystallised from chloroform-petroleum ether mixture, m.p. 201–3°.

Identification of the methylated sugars

The filtrate after above acid hydrolysis, was neutralised with Ag_2CO_3, filtered and the filtrate was evaporated to a syrup. The methylated sugars in the syrup (180 mg) were resolved by preparative paper chromatography [solvent system : butanol : water (azeotrope); spraying reagent : aniline hydrogen phthalate] and three spots corresponding to authentic samples 2,3,6-tri-*o*-methyl-D-glucose, 2,3,5-tri-*o*-methyl-L-arabinose and 2,3-di-*o*-methyl-L-arabinose respectively were found. The respective zones were eluted with water and evaporated under vacuum to syrups (Nos. 1, 2 and 3).

Identification of 2,3,5-*Tri*-o-*Methyl-L-Arabinose*

The syrup (No. 2) had $[\alpha]_D$—36° (water). The 2,3,5-tri-*o*-methyl-L-arabinose was characterised by its crystalline 2,3,5-tri-*o*-methyl-L-arabonamide[10,11], m.p. and mixed m.p. 134–36°.

Identification of 2,3-*di*-o-*methyl-L-arabinose*

The syrup (No. 3) had $[\alpha]_D$+88° (water). Its *p*-nitrobenzoyl derivative was prepared in the usual way[12] and the product was crystallised from ethanol, m.p. and mixed m.p. 150–51°.

Identification of 2,3,6-*Tri*-o-*Methyl-D-Glucose*

The syrup (No. 1) had $[\alpha]_D$+67° (water). Its *p*-nitrobenzoyl derivative was prepared in the usual way[13] and the product (2,3,6-tri-*o*-methyl-D-glucose-1,4-di-*p*-nitrobenzoate; m.p., and mixed m.p. 190–91°) was crystallised from ethanol.

Estimation of total sugars

Mollugocin A (100.6453 mg) was heated in a sealed tube with 8% methanolic sulphuric acid (25 ml) for 10 hr. Methanol was then evaporated by repeated addition of water and the precipitated genin was filtered quantitatively, washed free from acid, dried and weighed (51.5505 mg). The genin was found to be 51.22%.

The aqueous layer was neutralised by $BaCO_3$, precipitated $BaSO_4$ and unreacted $BaCO_3$ filtered off, and the volume concentrated. An aliquot of this solution was estimated for total sugar[7]. The result was concordant with 45.02% of total sugars in mollugocin A.

Enzymatic hydrolysis of mollugocin A

Mollugocin A (15 mg) was dissolved in 2 ml of *p*H 5 buffer [an aqueous 0.5*M* sodium acetate solution

(35.2 ml)+0.5 *M* acetic acid (14.8 ml) and *β-glucosiade* (Sigma Corp., St. Louis, MO) 15 mg was added to it. The mixture was allowed to stand overnight at 37°. The precipitate was filtered off and crystallised from aq. methanol m.p. 250°–52°, $[\alpha]_D$+59.3° (ethanol). It was identical with an authentic sample of mollugogenol A. The filtrate was evaporated under vacuum to a syrup and this on paper chromatography (solvent system : *n*-butanol : acetic acid : water = 4 : 1 : 1, v/v, spraying reagent : aniline hydrogen phthalate) showed a spot at the base line much polar than that of D-glucose and L-arabinose.

Oxidation of the methylated aglycone with CrO_3-acetic acid

The methylated aglycone (vide supra) was dissolved in glacial acetic acid (5 ml) and to this solution CrO_3 in acetic acid solution was added dropwise with constant stirring till the brown colour persisted. The mixture was then stirred for further 15 min., and kept overnight at the room temperature. The reaction product was then worked up in the usual way. The oxidised product gave positive Zimmerman's test for 3-keto group.

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