Phytochemistry, 1972, Vol. 11, pp. 1791 to 1795. Pergamon Press. Printed in England.

SAPONINS OF THE SEEDS OF *BASSIA LATIFOLIA*

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(*Received* 3 *September* 1971, *in revised form* 26 *October* 1971)

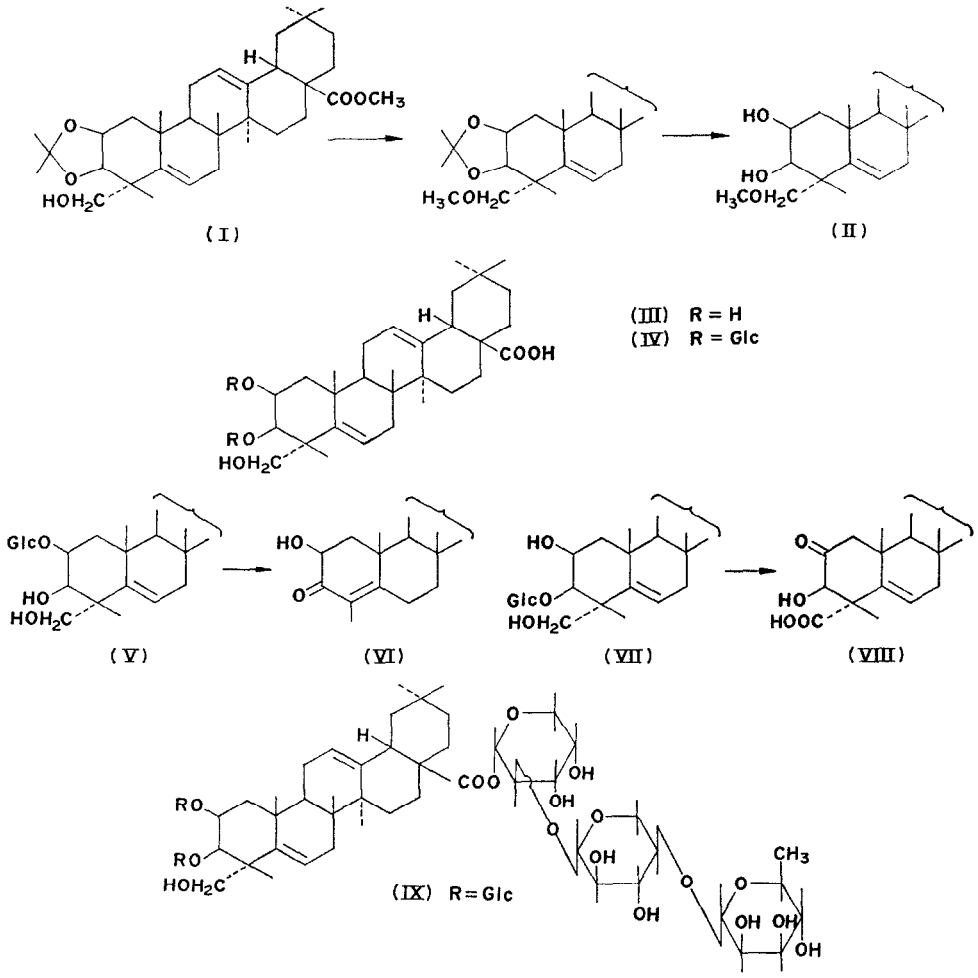
Abstract—Two saponins *A* and *B* have been isolated from the defatted seeds of *Bassia latifolia*. The structure of saponin A has been elucidated as 2,3-di-*O*-glucopyranoside of bassic acid (IV) and that of saponin *B* as rhamnopyranosyl 1→4 xylopyranosyl 1→2 arabopyranosyl 1→ OOC(28) of saponin *A* (IX).

Bassia latifolia Roxb. Is a large forest tree of Central India. The oil-free seed-cake is used as an insect poison.[1] Earlier investigation by Heywood *et al.*[2] showed the presence of bassic acid in the seeds. During our reinvestigation of this material we have been able to isolate two saponins in pure form and establish their structures. The crude saponin mixture obtained from the defatted seeds by standard methods, when chromatographed over silica gel, yielded two saponins *A* and *B*.

Saponin A

On acid hydrolysis it gave bassic acid (III), glucose and another substance, compound *X*. Saponin *A* reacted with diazomethane to give a monomethyl ester showing that the carboxy of the genin portion was free. Molecular weight determination by micro titration with standard alkali showed that saponin *A* was a diglucoside and this was supported by its elemental analysis. Saponin *A* was non-reducing; hence the anomeric hydroxyls should be involved in glycosidic linkages. Saponin *A* was permethylated by Hakomori's method,[3] the product hydrolysed and the sugar and genin portions examined separately. 2,3,4,6-Tetra-*O*-methylglucose was obtained as the only methylated sugar fragment; the two glucopyranose units must therefore be attached independently to two different hydroxyl groups of bassic acid. The partially methylated genin fragment resulting from the hydrolysis reacted with periodate, showing the presence of free vicinal hydroxyl groups. It was identified as the 23-methyl ether of methyl bassate (II) by direct comparison with an authentic sample which was prepared by methylating the acetonide of methyl bassate (I) and hydrolysing the product with acid. Therefore saponin *A* is 2,3-di-*O*-glucopyranoside of bassic acid. The configurations of the linkages were deduced by the application of Hudson's rule (using Klyne's[4] simplified method). The calculations are shown in Table 1. Hence both glucose units are β-linked. This was confirmed by hydrolysing the saponin with emulsin when D-glucose and bassic acid were formed. The structure of saponin *A* may therefore be represented by (IV).

[1] R. N. CHOPRA, *Indigenous Drugs of India*, p. 357, Dhur, India (1958).
[2] B. J. HEYWOOD, G. A. R. KON and L. L. WARE, *J. Chem. Soc.* 1124 (1939).
[3] S. HAKOMORI, *J. Biochem.* **55**, 205 (1964).
[4] W. KLYNE, *Biochem. J.* **47**, 41 (1950).



Compound X

Compound *X* referred to above was obtained in larger yield when saponin *A* was subjected to mild acid hydrolysis. It was acidic and it formed an ester with diazomethane. On further hydrolysis with 7% H_2SO_4 it gave glucose and bassic acid. Its hydrolysis was never complete since even after refluxing with 10% H_2SO_4 for 12 hr some starting material was still present. A similar difficulty in hydrolysis had been noted earlier in the case of alfalfa saponin.[5] Analyses of *X* and its methyl ester acetate showed that it was a monoglucoside. The NMR spectrum of the ester acetate showed the presence of six acetoxyl groups which supported the conclusion that it is a monoglucoside. Permethylation followed by acid hydrolysis gave 2,3,4,6-tetra-*O*-methylglucose as expected. Of the three hydroxyl groups which are available for the attachment of the sugar unit, that at C-23 was ruled out for the following reason. Compound *X* was treated with periodate and then hydrolysed with H_2SO_4, when bassic acid was formed. Had the sugar been attached to the hydroxyl at C-23 the genin would have been destroyed by periodate. This leaves structures (V) and (VII) as

[5] R. J. MORRIS, W. B. DYE and P. S. GISLER, *J. Org. Chem.* **26**, 1241 (1961).

TABLE 1. ROTATORY VALUES FOR SAPONIN A

Substance	$[\alpha]_D$ (°)	$[M]_D$ (°)
Observed values		
Saponin *A*	+35·0	+284
Bassic acid	+82·1	+399
$[M]_D$ contribution of glucose units		−115
Literature values		
2 × β-methyl-D-glucocopyranoside[6]		−124
2 × α-methyl-D-glucopyranoside[6]		+624

two alternatives. When saponin *X* methyl ester was subjected to Jones' oxidation and the product hydrolysed with acid, a complex mixture resulted. Under these conditions the product should be either (VI) or (VIII) if the structure of *X* is (V) or (VII) respectively.

The crude reaction mixture did not show any UV absorption for the presence of α-β-unsaturated carbonyl; on the other hand it was acidic. Examination of the alkali-soluble portion by TLC both before and after treatment with diazomethane showed that the spot which constituted the major compound before treatment disappeared and a new major spot with much higher R_f appeared. This indicated that the product was probably VIII but it could not be characterized further. Hence in compound *X* the glucose is most probably attached to the 3-position.

Saponin B

On complete hydrolysis with 7% H_2SO_4, it gave bassic acid, glucose, arabinose, xylose and rhamnose. It was inert towards diazomethane showing that the —COOH at C_{17} was blocked. When hydrolysed with 2% H_2SO_4, it gave, among other products, saponin *A*. Thus saponin *B* is composed of saponin *A* with additional sugars. When the ester linkage was cleaved with 15 % alkali and the prosaponin esterified with diazomethane, the product was identical with saponin *A* methyl ester. This clearly showed that saponin *B* carries arabinose, xylose and rhamnose units on its —COOH group and that the glucose units on ring A are not carrying any additional sugars nor is there any sugar attached to the OH at C-23. A quantitative estimation of sugars showed the ratio of xylose:arabinose:rhamnose to be 1:1:1; the complete hydrolysis of the glucose units being difficult, the estimation of this sugar was omitted. Saponin *B* was permethylated and hydrolysed. The partial methyl ethers of sugars were identified by paper chromatography (see Experimental) as 3,4-di-*O*-methylarabinose, 2,3-di-*O*-methylxylose, 2,3,4-tri-*O*-methylrhamnose and 2,3,4,6-tetra-*O*-methylglucose.

From the permethylation results, it is clear that rhamnose is an end sugar without any branching and is in pyranose form and that arabinose and xylose are inner sugars in the sugar chain and do not have any branching.

The mixture of partial methyl ethers obtained from permethylated saponin *B* was treated with periodate and then examined whereupon the partial methyl ether of arabinose was found to be missing; this confirmed that the arabinose derivative is its 3,4- and not its 2,3-di-*O*-methyl ether and that this sugar is in the pyranose form. The absence of any partial methyl ether of glucose other than 2,3,4,6-tetra-*O*-methylglucose together with the fact that there is no branching in the ester sugar chain showed that glucose is not present in the ester sugar chain.

It has already been shown that glucose, rhamnose and arabinose are in pyranose form.

[6] *Dictionary of Organic Compounds* (edited by I. HEILBRON, A. H. COOK, H. M. BUNBURY and D. H. HEY), Vol. 1, p. 826, Eyre & Spottiswoode, London (1965).

Xylose can be in either furanose or pyranose form, but since furanoxylose is very unusual among natural glycosides, it may be assumed that in saponin *B* the xylose unit is also in pyranose form.

In another experiment saponin *B* permethylate was reduced with $LiAlH_4$, the product hydrolysed and the sugar portion examined by paper chromatography. The spot due to 3,4-di-*O*-methylarabinose was missing showing that arabinose is the sugar directly linked to the $-COOH$.

Hence the structure of saponin *B* can be written as (IX), in which the D- or L-nature of the three sugars constituting the triose moiety in ester linkage at C_{28} of the aglycone is not completely established.

EXPERIMENTAL

For paper chromatography, Whatman No. 1 paper and the following solvents were employed: *n*-BuOH–HOAc–H_2O, 4:1:5 upper layer (A); *n*-BuOH–pyridine–H_2O, 6:4:3 (B); *n*-BuOH–pyridine–H_2O–benzene, 5:3:3:1 (upper layer) (C); *n*-BuOH–EtOH–H_2O, 5:1:4 upper layer (D); *n*-BuOH–EtOH–H_2O, 3:1:1 (E).

Isolation of saponins. The defatted seeds were extracted with alcohol and the extract concentrated to a syrup. It was macerated with ether and the residue was worked up for saponins by the *n*-BuOH method when a yellow powder was obtained. This was chromatographed over silica gel. Elution with $CHCl_3$–MeOH (92:8) gave saponin *A* and with $CHCl_3$–MeOH (4:1) gave saponin *B*.

Saponin A (IV). M.p. 216–218°(d), $[\alpha]_D$ +35·0° (*c*, 0·8 in pyridine) (Found: C, 62·1; H, 8·6. $C_{42}H_{66}O_{15}$ requires: C, 62·2; H, 8·2%).

Hydrolysis of (IV). It was refluxed with 7% H_2SO_4 in 80% aq. MeOH for 6 hr. After addition of water and removal of solvent the genin was filtered and chromatographed over silica gel. $CHCl_3$–MeOH (19:1) eluate gave a pure acid, m.p. > 310°, $[\alpha]_D$ +82·1° (*c*, 1·15 in pyridine) (Found: C, 74·1; H, 9·8. $C_{30}H_{46}O_5$ requires: C, 74·1; H, 9·5%); methyl ester, m.p. 222–224°, $[\alpha]_D$ + 60·0° (*c*, 1·11 in pyridine) (Found: C, 73·8; H, 9·6. $C_{31}H_{48}O_5$ requires: C, 74·4; H, 9·6%); acetyl methyl ester, m.p. 148–150°, $[\alpha]_D$ +68·2° (*c*, 1·24 in $CHCl_3$). Comparison with authentic samples of bassic acid and methyl bassate showed their identity (m.m.p., TLC and IR). $CHCl_3$–MeOH, (92:8) eluate in this chromatogram gave a small amount of substance *X*. The aqueous mother liquor when examined after neutralization showed the presence of glucose (PC, solvents A–C).

Permethylation of (IV) *and hydrolysis.* Sodium hydride dispersion in oil (50%, 20 mg) was added to a solution of (IV) (20 mg) in dimethyl sulphoxide (DMSO) (2 ml) and the mixture was kept at 80° for 1 hr. After cooling MeI (0·5 ml) was added and the mixture left overnight. The product was poured into ice-cold H_2O and extracted with $CHCl_3$. The syrup obtained on evaporation of the solvent was permethylated two more times and resulting product which was homogeneous according to TLC was hydrolysed using 7% aq. methanolic H_2SO_4. MeOH was removed after adding water. The precipitated genin was filtered and purified by chromatography over neutral alumina, m.p. 159–161°, $[\alpha]_D$ +61·3° (*c*, 1·20 in $CHCl_3$) (Found: C, 74·2; H, 9·5. $C_{32}H_{50}O_5$ requires: C, 74·7; H, 9·7%.) It reacted with periodate to give a carbonyl compound (TLC, spray DNPH) and it was identified as 23-methyl ether of methyl bassate (II) (described later) (m.m.p., TLC and IR). The aqueous mother liquor was neutralized ($BaCO_3$) and examined for methylated sugars when 2,3,4,6-tetra-*O*-methylglucose was identified (direct comparison with authentic sample by PC in solvent D).

Partial synthesis of 23-methyl ether of methyl bassate. To a solution of methyl bassate (150 mg) in dry acetone *p*-toluene sulphonic acid (1 mg) was added. After keeping for 4 hr at room temp. the product (acetonide (I)) was worked up and purified by column chromatography over neutral alumina, m.p. 206–207°, $[\alpha]_D$ +74·0° (*c*, 1·00 in $CHCl_3$) (literature m.p. 206–208°).[7] IR (KBr): 1170, 1156, 1096, 1047, 1036, 860 cm^{-1}.

The above acetonide was methylated using CH_3I and NaH in DMSO exactly as described under permethylation of (IV). The product (23-methyl ether of methyl bassate 2,3,-acetonide) after purification by column chromatography (neutral alumina) was crystallized from MeOH as flat prisms, m.p. 165–167°, $[\alpha]_D$ +86° (*c*, 1·00 in $CHCl_3$).

On heating with HCl at 80° for 1 hr the above product gave 23-methyl ether of methyl bassate, m.p. 160–162°, $[\alpha]_D$ +66·7° (*c*, 1·70 in $CHCl_3$) (Found: C, 74·2; H, 10·2; OCH_3, 11·7. $C_{32}H_{50}O_5$ requires: C, 74·7; H, 9·7; OCH_3(2), 12·1%). NMR values in $CDCl_3$: δ 3·65, 3H ($COOC\underline{H}_3$), δ 3·42, 3H ($CH_2OC\underline{H}_3$).

Enzymatic hydrolysis of (IV). (IV) (15 mg) in 10% aq. MeOH (3 ml) was treated with almond emulsion, a few drops of toluene added and the mixture kept at 37°. After 7 days the genin was worked up and found to be a mixture of saponin *A*, compound *X* and bassic acid (TLC); the sugar was glucose.

Preparation of compound X. (IV) was hydrolysed with 2% H_2SO_4 and the genin portion chromatographed over silica gel. Compound *X* was obtained in major amounts and bassic acid in minor amounts. Compound

[7] T. J. KING and J. P. YARDLEY, *J. Chem. Soc.* 4308 (1961).

X, m.p. 162–164°, $[\alpha]_D$ +60·4° (*c*, 1·40 in pyridine) (Found: C, 66·2; H, 9·0. $C_{36}H_{56}O_{10}$ requires: C, 66·7; H, 8·6%); methyl ester m.p. 215°(d), $[\alpha]_D$ +42·3° (*c*, 0·90 in pyridine); methyl ester acetate, m.p. 142–144°, $[\alpha]_D$ +37·4° (*c*, 1·10 in $CHCl_3$) (Found: C, 64·8; H, 8·0. $C_{49}H_{70}O_{16}$ requires: C, 64·2; H, 7·6%).

Hydrolysis of compound X. This was done using 7% H_2SO_4 in 80% aq. MeOH. The genin was extracted with Et_2O and chromatographed over silica gel. $CHCl_3$–MeOH (19:1) eluate gave bassic acid and $CHCl_3$–MeOH (92:8) gave compound *X*. The aqueous mother liquor contained glucose (PC in solvents A–C).

Periodate oxidation of compound X *and hydrolysis.* Compound *X* was treated with excess of $NaIO_4$ in MeOH for 48 hr at room temp. The product was worked up by extracting with *n*-BuOH and hydrolysed using 7% aq. H_2SO_4. The genin was identical with bassic acid.

Oxidation of compound X *methyl ester and hydrolysis.* This was done using Jones' reagent and the product was extracted with *n*-BuOH. The butanol extract was washed neutral and evaporated. The brown residue was hydrolysed with 7% H_2SO_4 in aq. methanol and the product extracted with ether. On removing the solvent a light reddish residue was obtained from which no homogeneous compound could be obtained.

Saponin B (IX). M.p. 212–214°, $[\alpha]_D$ −30° (*c*, 1·50 in pyridine) (Found: C, 54·1; H, 7·5. $C_{58}H_{98}O_{30}$ requires: C, 54·6; H, 7·7%).

Hydrolysis of (IX) *with* 7% H_2SO_4. This was done exactly as described under (IV) and the genin portion chromatographed over silica gel, when bassic acid and a small amount of compound *X* (TLC) were obtained. The aqueous mother liquor was neutralized ($BaCO_3$) and examined by PC in solvents A–C; glucose, arabinose, xylose and rhamnose were identified. The compound *X* was produced in greater yield when saponin *B* was hydrolysed with 2% H_2SO_4.

Hydrolysis of (IX) *with* 15% KOH. (IX) (50 mg) was heated with 15% aq. KOH (15 ml) at 80° for 45 min. After acidification it was extracted with *n*-BuOH, the butanol extract neutralized and evaporated. The residue was treated with ethereal CH_2N_2 and the product crystallized from MeOH–Et_2O. It was identical with saponin *A* methyl ester (m.p., m.m.p., TLC and IR).

Quantitative sugar estimation. A known quantity of (IX) was hydrolysed with Kiliani mixture (HOAc–HCl–H_2O, 7:2:11) at 100° for 6 hr in a sealed tube. The sugar portion was chromatographed on paper, sprayed with aniline hydrogen phthalate, the sugar spots eluted with 60% aq. HOAc and estimated colorimetrically.

Permethylation of (IX) *and hydrolysis.* This was carried out using CH_3I and NaH in DMSO and the product was hydrolysed with Kiliani mixture. The methylated sugars were identified by PC. Authentic 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-L-rhamnose and 2,3,4-tri-*O*-methyl-L-arabinose, were used as standards. The R_G values of the four spots obtained with solvent D were 1·01, 1·00, 0·72 and 0·59 (R_G of authentic 2,3,4,6-tetra-*O*-methyl-D-glucose is taken as 1) and the *RAr* values of the four spots obtained with E were 1·18, 1·17, 0·92 and 0·72 (*RAr* of authentic 2,3,4-tri-*O*-methyl-L-arabinose is taken as 1). The R_G values of the first three spots in D are, according to literature,[8] those of 2,3,4-tri-*O*-methyl-L-rhamnose, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-xylose; the first two spots also compared well with standard substances; the fourth and slow moving spot (R_G 0·59) could not be identified, but it cannot be due to 2,3-di-*O*-methyl-arabinose whose R_G according to literature[8] should be 0·64. Again, the first two spots in E are those of 2,3,4-tri-*O*-methylrhamnose and 2,3,4,6-tetra-*O*-methylglucose (direct comparison with authentic samples) and the spot with *RAr* 0·72 should be due to 3,4-di-*O*-methylarabinose whose *RAr* according to literature[9] should be 0·72. The fourth spot in E (*RAr* 0·92) is evidently derived from the xylose moiety.

When the mixture of partial methyl ethers was treated first with periodate and the product examined subsequently, the spot with R_G 0·59 in D and *RAr* 0·72 in E alone was absent.

$LiAlH_4$ *reduction of saponin* B *permethylate and hydrolysis.* Saponin *B* permethylate (15 mg) was reduced with $LiAlH_4$ (20 mg) in boiling tetrahydrofuran (5 ml) for 6 hr. After decomposition of excess of $LiAlH_4$ with moist EtOAc the product was acidified and extracted with EtOAc. The solvent was removed, the residue hydrolysed with Kiliani mixture and the sugar portion examined by PC in D. Only three spots with R_G values 1·01 (2,3,4-tri-*O*-methylrhamnose), 1·00 (2,3,4,6-tetra-*O*-methylglucose) and 0·72 (2,3-di-*O*-methylxylose) were obtained.

Acknowledgements—The authors are grateful to Professor T. R. Seshadri for his kind interest and the Indian Council of Medical Research and the Central Council for Research in Indian Medicine and Homoeopathy for financial assistance.

[8] E. LEDERER and M. LEDERER, *Chromatography* p. 249, Elsevier, New York (1957).
[9] S. C. WILLIAMS and J. K. N. JONES, *Can. J. Chem.* **45**, 225 (1967).

Key Word Index—Bassia latifolia seed; Sapotaceae; saponins; bassic acid glycosides.