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# CHEMICAL EXAMINATION OF THE ROOTS OF *TERMINALIA ARJUNA*—THE STRUCTURES OF ARJUNOSIDE III AND ARJUNOSIDE IV, TWO NEW TRITERPENOID GLYCOSIDES*

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**Key Word Index**—*Terminalia arjuna*; Combretaceae; arjunoside III; arjunoside IV; triterpenoid glycosides.

**Abstract**—The non-phenolic fraction of the alcoholic extract of the root bark of *Terminalia arjuna* yielded two new triterpenoid glycosides, arjunoside III and arjunoside IV in addition to arjunglucoside I and arjunetin. The structure of arjunoside III was established as the 28-$\beta$-D(+)-glucuronopyranoside of arjunic acid by a study of its chemical and spectroscopic ($^1$H and $^{13}$C NMR) data. Arjunoside IV was shown to be the 3-$O$-$\alpha$-L(−)-rhamnoside of arjunic acid. Leucocyanidin, ellagic acid and gallic acid have been isolated from the phenolic part of the root extract.

## INTRODUCTION

The isolation and structure elucidation of two new triterpenoid glycosides, arjunoside I and arjunoside II were recently reported from the ethyl acetate extract of the root bark of *T. arjuna* (Combretaceae) [1]. Both of these compounds are *O*-glycosides of arjunic acid (**1**), the former being the 3-$O$-$\beta$-D(+)-galactoside (**2**) and the latter tentatively being established as its D(+)-glucosyl 2-deoxy-$\alpha$-L(−)-rhamnoside (**3**). The alcoholic extract of the same roots furnished two more new glycosides of arjunic acid (**1**). These are arjunoside III and arjunoside IV whose structures have now been established as the 28-$\beta$-D(+)-glucuronopyranoside (**5**) and 3-$O$-$\alpha$-L(−)-rhamnopyranoside (**4**) respectively, of arjunic acid.

## RESULTS AND DISCUSSION

The alcohol extract was separated into phenolic and non-phenolic fractions by precipitation with basic lead acetate. The phenolic fraction from the lead salt yielded leucocyanidin, ellagic acid and gallic acid. The non-phenolic fraction yielded arjunetin [2], arjunglucoside I [3] and two new glycosides arjunoside III and arjunoside IV.

Arjunoside III (**5**), $C_{36}H_{56}O_{11}$, mp 272–274°, $[\alpha]_D$ + 20.30° was recognized as a triterpene glycoside from its colour reactions (pink colour in the LB test, yellow with TNM and violet in the Molisch test). It was transparent in the UV region and showed peaks in its IR spectrum at 3400–3300 cm$^{-1}$ (*br*) for a polyhydroxy system, at 1705 and 1725 cm$^{-1}$ for acid and ester carbonyls and at 1660 and 850 cm$^{-1}$ for a trisubstituted double bond. It gave a monomethylester, $C_{37}H_{58}O_{11}$, mp 210–213°, $[\alpha]_D$ + 15° with $CH_2N_2$, a pentaacetate, $C_{46}H_{66}O_{16}$, mp 192–194°, $[\alpha]_D$ + 11.11° with $Ac_2O$–pyridine. The pentaacetate gave a methylesteracetate, $C_{47}H_{68}O_{16}$, mp 180–182°, $[\alpha]_D$ + 8.2°.

Unlike arjunosides I and II, arjunoside III behaved as an ester glycoside with an additional carboxyl group. However, like the former two, it is a glycoside of arjunic acid which it furnished during 8% alkali hydrolysis. Arjunic acid (**1**) [4] being the aglycone, the second carboxyl group must have obviously been present in the sugar moiety.

Paper chromatography of the aqueous hydrolysate from arjunoside III after careful neutralization and concentration under vacuum showed a single spot of $R_f$ value 0.315. This was very high for a hexose carboxylic acid with a free –COOH group. However, the observed $R_f$ value corresponded to the lactone of glucuronic acid [5]. That the lactone was glucuronolactone was proved by co-chromatography with an authentic sample. In the glycoside it should have been present as glucoronic acid with the carboxyl group free, as it formed the methyl ester. Such exclusive formation of glucuronic acid lactone was earlier noticed during its preparation by the hydrolysis of bornyl glucuronide [6] under similar conditions.

During periodate oxidation, arjunoside III consumed three moles of periodate during 24 hr suggesting the presence of three *trans*-glycol units. Two of them were present in D(+)-glucuronic acid, the third one was inferred to be in the 2, 3 vicinal dihydroxy system of arjunic acid. The foregoing information suggested that arjunoside III was the 28-glucuronide of arjunic acid.

The $\beta$-glycosidic linkage of D(+)-glucuronic acid with arjunic acid was arrived at by a study of enzymatic hydrolysis and application of molecular rotation data by Klyne's rule [7]. Hydrolysis of arjunoside III was effected by $\beta$-D-glucuronidase in acetic acid–NaOAc buffer indicating the nature of its sugar and its linkage. In the hydrolysate two spots were identified, one corresponding to D-glucuronic acid ($R_f$ = 0.12) [5] and the other to its lactone ($R_f$ =

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0.32) [5]. The difference ($\Delta C = +37°$) in molecular rotation of arjunoside III (+134°) and arjunic acid (+98%) is nearer to the molecular rotation of the $\beta$-D(+)-methyl glucuronopyranoside (−82°) [8] but not to that of $\alpha$-D(+)-methyl glucuronopyranoside (+265°) [8] adding further support to the $\beta$-glycosidic nature of arjunoside III.

The $^1$H NMR spectrum of arjunoside III acetate (**5b**) showed seven tertiary methyls between $\delta$ 0.68 and 1.22, five acetoxyls between 2.00 and 2.04, a broad singlet at 3.05 for the 18$\beta$-H, another at 3.3 for the 19$\beta$-H and two doublets were seen at 3.75 with $J_{4',5'} = 6$ Hz and at 4.70 with $J = 10$ Hz assignable to the 5'-H and 3$\alpha$-H, respectively. A broad multiplet between 5.1 and 5.5 accounted for the other hydrogens at 2', 3', 4', 12 and 2$\beta$-H. The anomeric proton was clearly discernible at 5.56 as a doublet ($J = 8$ Hz). The chemical shift, as well as the coupling constant, were reminiscent of the anomeric proton noticed in other ester glycosides like arjunetin [2], arjunglucosides I [3], II [3] and III [9] confirming the ester glycosidic nature of arjunoside III as well as its $\beta$-glycosidic linkage.

The $^{13}$C NMR spectrum of arjunoside III and arjunic acid were determined and the chemical shifts of all the carbons were assigned on the basis of comparison with the data reported for other glycosides [9].

Arjunoside III showed very clearly two low-field carbons at $\delta$ 175.66 and 181.49 accounting for the two carbonyls, one of the ester and the other of the free carboxyl group. The anomeric carbon appeared at 93.99 which is reminiscent of the one observed [9] in arjunetin and arjunglucoside III. It has been noticed in the literature [10] that the anomeric carbon of the *O*-glycosides appears around 100 ppm irrespective of the nature of the sugar unit, which thus can be distinguished readily from the anomeric carbon of the ester glycosides that appears around 95 ppm.

The foregoing account thus establishes the structure of arjunoside III as the 28$\beta$-D(+)-glucuronopyranoside of arjunic acid (**5**).

Arjunoside IV (**4**), $C_{36}H_{58}O_9$, mp 260–265°, $[\alpha]_D$ −10° was recognized from preliminary colour tests as a triterpenoid glycoside. Its IR spectrum showed peaks at 3600–3400 (–OH), 1650 and 850 (–C=C–H) and 1710 (–COOH) cm$^{-1}$.

Arjunoside IV differed from arjunoside III but behaved like an *O*-glycoside like arjunosides I and II. However, it gave the same genin, arjunic acid (**1**), on acid hydrolysis. The aqueous hydrolysate showed a single spot on paper chromatography ($R_f = 0.37$, $R_g = 0.30$) corresponding to L(−)-rhamnose, the identity of which was proved by co-chromatography with an authentic sample, Arjunoside IV was thus a rhamnoside of arjunic acid linked most probably by its 3$\beta$-hydroxyl from biogenetic considerations.

The nature of the glycosidic linkage might be reasonably assumed as $\alpha$-consistent with the observation that the L-sugars are involved in $\alpha$-glycosidic linkages [11]. It resisted hydrolysis with $\beta$-D-glucosidase which provides some evidence for the absence of a $\beta$-linkage. Positive evidence could not be furnished because of non-availability of the specific enzyme.

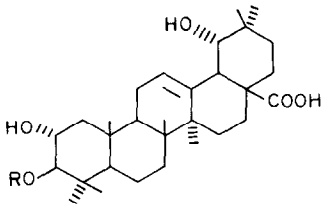
The difference ($\Delta C = -161°$) between the molecular rotation of arjunoside IV (−63°) and that of arjunic acid (+98°) was nearer to the molecular rotation of $\alpha$-methyl L-rhamnoside [12] (−111°) but not to that of $\beta$-methyl L-rhamnoside [12] (+170°) adding

Table 1. $^{13}$C NMR chemical shift data of arjunic acid and arjunoside III*

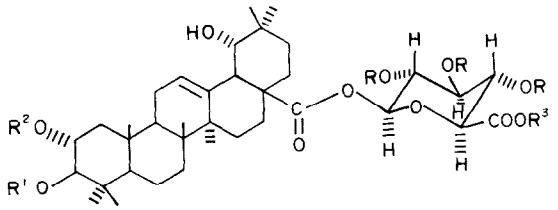
| Carbon number | Arjunic acid | Arjunoside III | Carbon number | Arjunic acid | Arjunoside III |
|---|---|---|---|---|---|
| C-1 | 47.17 | 47.23 | C-19 | 80.02 | 79.96 |
| C-2 | 67.07 | 67.05 | C-20 | 36.87 | 36.81 |
| C-3 | 82.19 | 82.19 | C-21 | 28.42 | 28.24 |
| C-4 | 38.81 | 38.80 | C-22 | 34.79 | 34.67 |
| C-5 | 54.83 | 54.83 | C-23 | 27.12 | 27.95 |
| C-6 | 16.94 | 16.14 | C-24 | 16.76 | 16.58 |
| C-7 | 32.38 | 31.70 | C-25 | 16.11 | 16.14 |
| C-8 | 38.95 | 39.01 | C-26 | 16.94 | 16.91 |
| C-9 | 47.17 | 47.23 | C-27 | 28.68 | 27.95 |
| C-10 | 38.54 | 38.48 | C-28 | 179.00 | 175.66 |
| C-11 | 23.16 | 23.19 | C-29 | 28.68 | 28.62 |
| C-12 | 122.06 | 122.19 | C-30 | 24.42 | 24.39 |
| C-13 | 143.37 | 143.05 | C-1' | — | 93.99 |
| C-14 | 41.89 | 41.83 | C-2' | — | 69.42 |
| C-15 | 28.42 | 28.24 | C-3' | — | 72.27 |
| C-16 | 24.04 | 24.04 | C-4' | — | 76.56 |
| C-17 | 46.64 | 45.12 | C-5' | — | 77.50 |
| C-18 | 43.12 | 43.00 | C-6' | — | 181.49 |

*$^{13}$C FT NMR spectra were measured at 25.05 MHz using DMSO-$d_6$ $CDCl_3$ solutions.

FT conditions: spectral width, 5 kHz; pulse flipping angle 60°; pulse repetition time, 1.0 sec; number of data points 4 K; number of transients 5–40 K; chemical shifts are expressed in $\delta$ (ppm) downfield from int. TMS.



**1** R = H
**2** R = β-D(+)-galactose
**3** R = β-D(+)-glucosyl-L(−)-2-deoxyrhamnose
**4** R = α-L(−)-rhamnopyranose



**5** $R^1 = R^2 = R^3 = R = H$
**5a** $R^1 = R^2 = R = H$, $R^3 = Me$
**5b** $R^1 = R^2 = R = Ac$, $R^3 = H$
**5c** $R^1 = R^2 = R = Ac$, $R^3 = Me$

support for the presence of the α-glycosidic linkage.

From the foregoing evidence arjunoside IV might be described reasonably as the 3-*O*-α-L(−)-rhamnopyranoside of arjunic acid (**4**). However, it is evident that the structure needs further characterization by means of spectral data and preparation of derivatives which were not possible for lack of material.

Chemical examination of the root bark of *T. arjuna* revealed the presence of four new glycosides of arjunic acid of which three are 3-*O*-glycosides and one is its 28-D(+)-glucuronide. The presence of *O*-glycosides has not been reported from any other part of the plant.

## EXPERIMENTAL

The root bark powder (10 kg), after successive extraction with *n*-hexane, $CHCl_3$ and EtOAc was extracted with EtOH. The reddish-brown EtOH extract (2 l.) was treated with a soln of basic lead acetate until no more yellow ppt. was formed. The filtrate was freed from lead by passing $H_2S$ and removing the black lead sulphide formed. It was then concentrated under vacuum to leave a red gum (non-phenolic fraction).

The basic lead salt was suspended in EtOH and decomposed by $H_2S$ and filtered. The filtrate after concentration under vacuum gave a brownish-yellow (phenolic) fraction.

*Non-phenolic fraction.* The non-phenolic residue was chromatographed over Si gel (100–200 mesh) to yield four compounds (Table 2).

*Identification of arjunoside* III (**5**). This compound crystallized from $CHCl_3$–MeOH as colourless needles, mp 272–274°, $[\alpha]_D + 20.3°$ (*c*, 1.75, MeOH). (Found: C, 65.00; H, 8.45. $C_{36}H_{56}O_{11}$ requires: C, 65.06; H, 8.43%.) IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3400–3300, 1705, 1725, 1660, 820.

*Acetate of arjunoside* III (**5b**). Arjunoside III (50 mg) in dry pryidine (5 ml) and $Ac_2O$ (4 ml) was heated on a steam-bath for 62 hr. The acetate crystallized from $CHCl_3$–MeOH as colourless needles, mp 192–194°, $[\alpha]_D + 11.11°$ (*c*, 0.81, $CHCl_3$). (Found: C, 63.06; H, 7.50. $C_{46}H_{66}O_{16}$ requires: C, 63.16; H, 7.55%.) IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3600, 1740–1720, 1600, 1250, 850; $^1H$ NMR (90 MHz, $CDCl_3$): δ 0.68, 0.92, 1.04, 1.22 (21 H, all *s*, 7 × *tert*.Me), 2.0–2.02 (15 H, two *s*, 5 × $OCOCH_3$), 3.05 (1 H, *br.s*, $W_{1/2} = 5$ Hz, 18β-H), 3.30 (1 H, *br.s*, $W_{1/2} = 4.4$ Hz, 19β-H), 4.20 (1 H, *d*, $J = 6$ Hz, 5′-H), 4.70 (1 H, *d*, $J = 10$ Hz, 3α-H), 5.1–5.5 (5 H, *br.m*, 12, 2′, 3′, 4′, 2β-H), 5.56 (1 H, $J = 8$ Hz, 1′-H).

*Methyl ester of arjunoside* III (**5a**). Arjunoside III (**5**) (30 mg) in dry $Et_2O$ was treated with excess of ethereal $CH_2N_2$ at 0° and this mixture kept overnight. The ester crystallized from $CHCl_3$–MeOH as colourless plates, softened at 192°, melted between 210–213°, $[\alpha]_D + 15.2°$ (*c*, 0.65, MeOH). (Found: C, 65.40; H, 8.60. $C_{37}H_{58}O_{11}$ requires: C, 65.48; H, 8.55%.) IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3600–3200, 1735, 1640, 830.

*Methyl ester acetate of arjunoside* III (**5c**). The pentaacetate of arjunoside III (**5b**) (15 mg) in dry $Et_2O$ was treated with excess of ethereal $CH_2N_2$ at 0° and the mixture kept overnight. The methyl ester acetate crystallized from $CHCl_3$–MeOH as colourless needles, mp 180–182°, $[\alpha]_D + 8.2°$ (*c*, 0.6, $CHCl_3$). (Found: C, 63.44; H, 7.52. $C_{47}H_{68}O_{16}$ requires: C, 63.51; H, 7.66%.) IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3500, 1740, 1660, 1245, 850.

*Acid hydrolysis of arjunoside* III. Compound **5** (20 mg) was refluxed with 8% methanolic $H_2SO_4$ (10 ml) for 6 hr. The genin, mp 335–337° was identified as arjunic acid (mmp and IR). The hydrolysate showed a single spot on PC ($R_f = 0.315$, *n*-BuOH–HOAc–$H_2O$, 4:1:5) which corresponded to glucuronic acid lactone.

*Alkali hydrolysis of arjunoside* III. Compound **5** (20 mg) was refluxed with 8% methanolic KOH (5 ml) for 6 hr. The hydrolysate showed two spots ($R_f = 0.12$, 0.32, *n*-BuOH–HOAc–$H_2O$, 4:1:5) on PC which corresponded to glucuronic acid and its lactone respectively.

Table 2. Column chromatography of the EtOH extract of *T. arjuna* root bark

| Fractions (500 ml) | Eluent | Yield (mg) | mp | $[\alpha]_D$ | Molecular formula | Identity |
|---|---|---|---|---|---|---|
| 1–20 | $CHCl_3$ | 100 | — | — | — | Mixture |
| 21–40 | $CHCl_3$–MeOH (90:10) | 600 | 272–274° | +20.23° | $C_{36}H_{56}O_{11}$ | Arjunoside III |
| 41–45 | $CHCl_3$–MeOH (90:10) | 20 | 260–265° | −10° | $C_{36}H_{58}O_9$ | Arjunoside IV |
| 46–50 | $CHCl_3$–MeOH (90:10) | 100 | 238–240° | +20° | $C_{36}H_{58}O_{10}$, $3H_2O$ | Arjunetin |
| 51–62 | $CHCl_3$–MeOH (85:15) | 30 | 130–232° | +13° | $C_{36}H_{58}O_{11}$, $H_2O$ | Arjunglucoside I |

*Enzymatic hydrolysis of arjunoside* III. Arjunoside III (10 mg) in NaOAc–AcOH buffer, pH 5.23 (3 ml) and β-D-glucuronidase (1 mg) were kept in an incubator at 37° for 10 days. Only two spots ($R_f = 0.12$, 0.32, *n*-BuOH–AcOH–$H_2O$, 4:1:5) were noted on PC after the first, second and tenth day.

*Periodate oxidation of arjunoside* III. Arjunoside III (20 mg) was dissolved in EtOH and treated with 0.3% aq. $KIO_4$. Aliqout portions removed after 2-, 4-, 24- and 36-hr intervals were titrated with 0.02 N sodium thiosulphate and found to consume 0.2, 0.2, 2.6 and 2.6 moles, respectively.

*Identification of arjunoside* IV (**4**). This material crystallized from $CHCl_3$–MeOH as colourless needles, mp 260–265°, $[\alpha]_D - 10°$ (*c*, 0.8, MeOH). (Found: C, 68.00; H, 9.04. $C_{36}H_{58}O_9$ requires C, 68.14; H, 9.15%.) IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3600–3400, 1710, 1650, 850.

*Acid hydrolysis of arjunoside* IV. Arjunoside IV (10 mg) was refluxed with 8% methanolic $H_2SO_4$ (10 ml) for 6 hr. The genin, mp 335–337°, was identified as arjunic acid. The hydrolysate ($R_f = 0.37$, *n*-BuOH–AcOH–$H_2O$, 4:1:5; $R_g$ = 0.30, *n*-BuOH–EtOAc–$H_2O$, 5:1:4) which was identified as L(−)-rhamnose by co-PC.

*Attempted enzymatic hydrolysis of arjunoside* IV. Arjunoside IV (**4**) (5 mg) in NaOAc–AcOH buffer of pH 5.23 (1 ml) and β-D-glucosidase (1 mg) was kept in an incubator at 37° for 10 days. No hydrolysis took place and no spot could be observed on PC even after the tenth day.

*Phenolic fraction.* The light yellowish-brown phenolic fraction was concentrated under red. pres. at 40–50° and was extracted with EtOAc. The mother liquor gave a solid after evapn, mp > 360° (60 mg), which was found to be leucocyanidin. The residue (300 mg) was adsorbed in Si gel (100–200 mesh) and chromatographed to give two compounds, gallic acid, mp 237–238° (30 mg) and ellagic acid, mp > 360° (30 mg).

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## REFERENCES

1. Anjaneyulu, A. S. R. and Rama Prasad, A. V. (1982) *Indian J. Chem.* (in press).
2. Ramachandra Row, L., Murty, P. S. N., Subba Rao, G. S. R., Sastry, C. S. P. and Rao, K. V. J. (1970) *Indian J. Chem.* **8**, 772.
3. Honda, T., Murae, T., Tsuyuki, T., Takahashi, T. and Sawai, M. (1976) *Bull. Chem. Soc. Jpn.* **49**, 3213.
4. Ramachandra Row, L., Murty, P. S. N., Subba Rao, G. S. R., Sastry, C. S. P. and Rao, K. V. J. (1970) *Indian J. Chem.* **8**, 716.
5. Patridge, M. and Westall, R. G. (1948) *Biochem. J. Lond.* **42**, 238.
6. Pryde, T. and Williams, R. T. (1933) *Biochem. J., Lond.* **27**, 1205.
7. Klyne, W. (1950) *Biochem. J., Lond.* **47**, 4.
8. Heilbron, I. (1965) *Dictionary of Organic Compounds*, Vol. 3, p. 1529. Eyre & Spottiswoode, London.
9. Tsuyuki, T., Hamada, Y., Honda, T., Takahashi, T. and Matsuhita, K. (1979) *Bull. Chem. Soc. Jpn.* **52**, 3127.
10. Boyd, J. and Turvey, J. R. (1978) *Carbohydr. Res.* **61**, 223.
11. Hirst, E. L. and Jones, J. K. N. (1949) *Discuss. Faraday Soc.* **7**, 268.
12. Heilbron, I. (1965) *Dictionary of Organic Compounds*, Vol. 1, p. 826. Eyre & Spottiswoode, London.