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NAPHTHAQUINONES OF *ARNEBIA NOBILIS**

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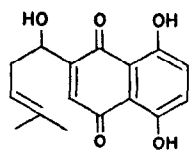
Abstract—Silica gel chromatography of the fraction from *Arnebia nobilis* possessing antibiotic and anticancer activities has yielded three new naphthaquinones, 5,8-dihydroxy-2-(1′-β,β-dimethylacryloxy-4′-methylpentyl)-1,4-naphthaquinone, 5,8-dihydroxy-2-(4′-hydroxy-4′-methylpentyl)-1,4-naphthaquinone and 2-(1′-acetoxy-4′-hydroxy-4′-methylpentyl)-5,8-dihydroxy-1,4-naphthaquinone, along with alkannin [5,8-dihydroxy-2-(1′-hydroxy-4′-methylpent-3′-enyl)-1,4-naphthaquinone], 5,8-dihydroxy-2-(1′-β,β-dimethylacryloxy-4′-methylpent-3′-enyl)-1,4-naphthaquinone and 2-(1′-acetoxy-4′-methylpent-3′-enyl)-5,8-dihydroxy-1,4-naphthaquinone, which were reported earlier.

INTRODUCTION

Arnebia nobilis Rachinger (Hindi: Ratanjot) is one of four species of the genus (Boraginaceae) occurring in Northern India. Investigations on the antibiotic fraction from this plant led to the isolation of four naphthaquinones designated as *A-1*, *A-2*, *A-3* and *A-4*.[1] *A-4* was identified as alkannin [5,8-dihydroxy-2-(1′-hydroxy-4′-methylpent-3′-enyl)-1,4-naphthaquinone] (I)[2] and *A-3* and *A-1* characterized as alkannin acetate [2-(1′-acetoxy-4′-methylpent-3′-enyl)-5,8-dihydroxy-1,4-naphthaquinone] and alkannin β,β-dimethylacrylate [5,8-dihydroxy-2-(1′-β,β-dimethylacryloxy-4′-methylpent-3′-enyl)-1,4-naphthaquinone] respectively. As a consequence of a programme for screening Indian plants over a wide range of biological activities,[3] ethanol extracts of *Arnebia nobilis* were found to possess



I

anticancer activity. This activity was also associated with the naphthaquinone fraction and alkannin β,β-dimethylacrylate (*A-1*) and alkannin acetate (*A-3*) have now been 'confirmed' for activity against Walker carcinosarcoma in rats.[4] This paper describes the isolation and fractionation of the naphthaquinones of *Arnebia nobilis* and the chemical characterization of *A-2* and of *A-5* and *A-6*, two naphthaquinones not reported in our earlier communication.[1]

* Communication No. 1541 from Central Drug Research Institute.

[1] Y. N. SHUKLA, J. S. TANDON, D. S. BHAKUNI and M. M. DHAR, *Experientia* **25**, 357 (1969).

[2] H. BROCKMAN, *J. Liebigs Ann. Chem.* **521**, 1 (1936); R. H. THOMSON, *Naturally Occurring Quinones*, p. 111, Butterworths, London (1957); A. C. JAIN and S. K. MATHUR, *Bull. Natn. Inst. Sci. India* **28**, 52 (1965).

[3] D. S. BHAKUNI, M. L. DHAR, M. M. DHAR, B. N. DHAWAN and B. N. MEHROTRA, *Indian J. Exptl. Biol.* **7**, 250 (1969).

[4] I. S. MATHUR and S. K. GUPTA, in press.

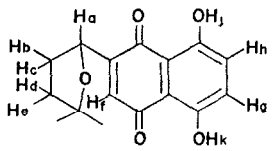
RESULTS AND DISCUSSION

Chromatography of the hexane extractable material from the air-dried roots on a silica gel column yielded six dark red coloured compounds. *A-1* and *A-3* were eluted with a hexane–benzene mixture, *A-4* with benzene and *A-2*, *A-5* and *A-6* with a benzene–chloroform mixture.

A-2

A-2, m.p. 92–94° analysed for $C_{21}H_{24}O_7$ and gave a deep blue solution with alkali. It had λ_{max} 223 (25,640), 280 (9050), 486 (6240) and 516 (7110) nm(ϵ).* Its IR spectrum had ν_{max} 1706 and 1144 cm^{-1} (α,β-unsaturated ester) 3284, 2955 and 1410 cm^{-1} (free and bonded OH), 1608 cm^{-1} (bonded carbonyl) 1372 and 1353 cm^{-1} $(CH_3)_2C$—) and 1600, 1560, 1450 and 760 cm^{-1} (aromatic nature).

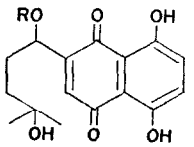
On hydrolysis with N-NaOH at 40° for 1 hr in an atmosphere of N_2, *A-2* yielded two dark red crystalline compounds *A-2A* m.p. 91° and *A-2B* m.p. 162° and an acid m.p. 65–66°. The acid had λ_{max} 216 (11,500) nm(ϵ) and was identified as β,β-dimethylacrylic acid by GLC by comparison with an authentic sample. It is not tiglic acid as suggested earlier.



II

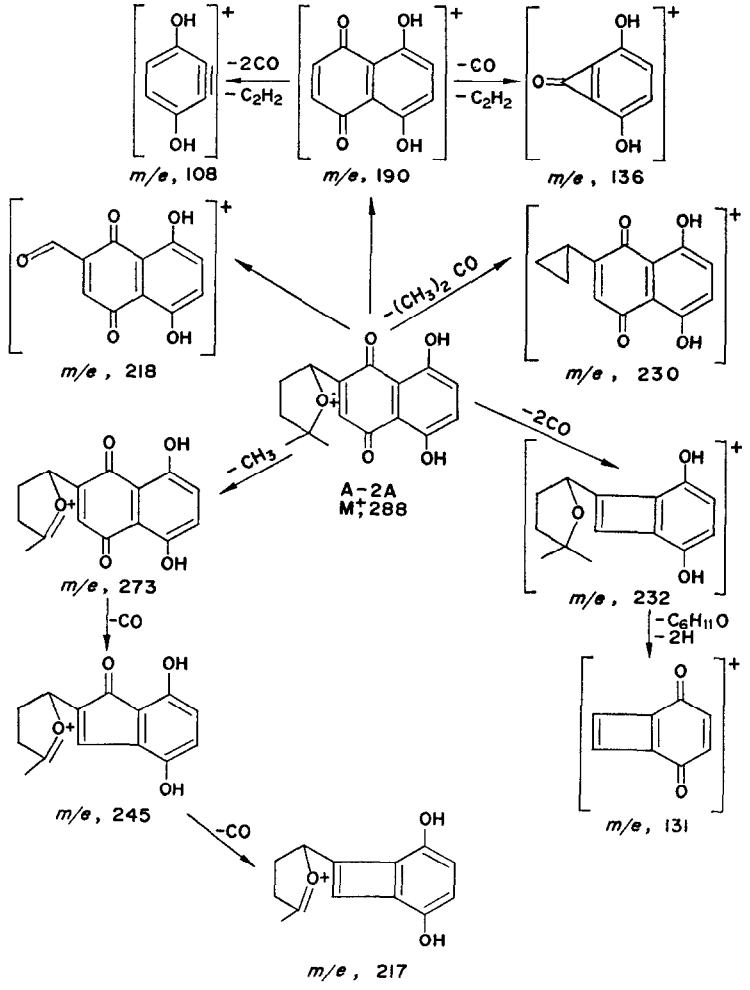
A-2A, $C_{16}H_{16}O_5$ (M^+ 288) was assigned the structure II. *A-2A* had λ_{max} 223 (12,200), 280 (8300), 484 (6300) and 508 (7000) nm (ϵ), no ester C=O stretching absorption in its IR spectrum and formed a diacetate m.p. 118–120°. Its NMR spectrum indicated the presence of 16 protons in the molecule. Deshielded singlets of 3 protons each at 8·62 and 8·68 τ appeared to be due to a gem dimethyl group attached to an oxygen atom. An octet centred at 4·98 τ was possibly due to Ha, the proton α to the quinone ring, which can couple with two non-equivalent methylene protons and the proton on the quinone ring. Signals for the four methylene protons Hb, Hc, Hd, He were seen as a multiplet for one of the protons centred at 7·80 τ and a multiplet for the remaining three at 8·22 τ and suggested the presence of a tetrahydrofuran system in the molecule. A two proton singlet at 2·90 τ was assignable to two equivalent aromatic protons (Hg, Hh) and a one proton doublet at 2·94 τ (J, 1·1 Hz) to the quinone proton (Hf). A two proton singlet at −2·35 τ disappeared on D_2O shake and could be assigned to two phenolic protons (Hj, Hk).

The base peak in the mass spectrum of *A-2A* is the molecular ion peak (M^+ 288). The formation of ions that most probably originate from the loss of an acetone residue (M^+ −58) and of an isopentylene residue (M^+ −70) support the existence of a tetrahydrofuran ring



III

* UV spectra determined in EtOH, IR spectra in KBr and 60 Mc/s, NMR spectra in $CDCl_3$ with TMS as internal standard.



SCHEME 1. FRAGMENTATION OF COMPOUND *A-2A*.

system in the molecule. The ions that are possibly responsible for these and the significant peaks *m/e* 273, 255, 245, 232, 217, 190, 136·5, 131, 108, 91 and 69 are depicted (Scheme 1). The fragment *m/e* 273 also gave rise to a doubly charged ion *m/e* 136·5.

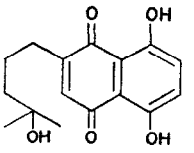
A-2B, the second naphthaquinone obtained on aklaline hydrolysis of *A-2*, analysed for $C_{16}H_{18}O_6$ (M^+ 306), had λ_{max} 225, 280, 486 and 508 nm and ν_{max} 3350, 3250, 2972, 1611, 1600, 1572, 1456, 1210 and 760 cm^{-1}. On treatment with HCl in dry benzene, *A-2B* lost a molecule of water and yielded *A-2A*. *A-2B* is therefore the hydroxyalkannin III (R=H). Consideration of the NMR spectrum of the parent *A-2* (see below) excludes the possibility of the secondary hydroxyl being on the β-carbon atom of the side chain. On electron impact, the molecular ion loses a molecule of water and subsequently fragments similarly to *A-2A* and alkannin (*A-4*).

With the characterisation of *A-2A* and *A-2B* and the identification of β,β-dimethylacrylic acid as the products of alkaline hydrolysis of *A-2*, it follows that *A-2* is a β,β-dimethylacrylic ester of *A-2B*. That the secondary hydroxyl and not the tertiary hydroxyl is

esterified follows from an examination of its NMR spectrum. The chemical shift of the secondary hydroxyl proton (—CH—OH) is at 5·10 τ in the spectrum of alkannin (*A-4*). Esterification would be expected to result in a downfield shift of about 60 Hz. In the spectra of alkannin acetate (*A-3*), alkannin β,β-dimethylacrylate (*A-1*) and of *A-2*, the chemical shifts of the signals for this proton are 4·04, 3·98 and 3·98 τ respectively, indicating that the hydroxyl esterified in *A-2* is the secondary hydroxyl. In addition, the NMR spectrum of *A-2* had a sharp singlet at 8·78 τ for six protons, assignable to the side chain gem methyls located on a carbon atom having a tertiary hydroxyl. A 4 proton multiplet around 8·30 τ was assignable to the two side chain methylenes and the tertiary hydroxyl proton gave rise to a singlet at 8·42 τ that disappeared on shaking with D_2O. The rest of the spectrum was similar to that of alkannin β,β-dimethylacrylate (*A-1*). Further confirmation for the structure of *A-2* was obtained when it was found that it is converted to alkannin β,β-dimethylacrylate by tosylation followed by treatment with Na_2CO_3.

A-5

A-5, m.p. 111–2° analysed for $C_{16}H_{18}O_5$ (M^+ 290) and had similar UV and IR spectra to alkannin, λ_{max} 222 (11,900), 280 (8900), 486 (5800) and 516 (6380) nm (ϵ) and ν_{max} 3567, 3050, 2950, 1610, 1572, 1467, 1220, 1130 and 770 cm^{-1}. Its NMR spectrum, however, had a significant difference. The signal for the quinone ring proton showed up as a triplet at 3·06 τ (*J*, 1·1 Hz) and not as a doublet as in the spectra of the other naphthaquinones from this plant. This suggested the presence of a methylene carbon α to the quinone ring and the structure IV for *A-5*. This structure is supported by the other signals in the NMR spectrum of *A-5*: six proton singlet at 8·76 τ assignable to $(CH_3)_2C$—OH, a two proton multiplet at 7·35 τ assignable to a CH_2 α to the quinone ring and a four proton multiplet around 8·4 τ assignable to two side chain CH_2. Two equivalent aromatic protons give rise to a singlet at 2·75 τ and singlets at −2·70 and −2·55 τ and at 8·52 τ, which disappear on D_2O shake, are assignable to the two phenolic and hydroxyl proton respectively.



IV

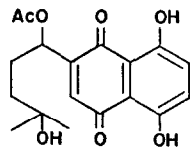
A-6

A-6, m.p. 88–90°, $C_{18}H_{20}O_7$ (M^+, 348) had λ_{max} 223 (13,500), 280 (7160), 486 (3840), 514 (4350) nm (ϵ) and ν_{max} 3450, 2962, 1735, 1612, 1600, 1565, 1455, 1410, 1210 and 760 cm^{-1}. The mass spectrum of *A-6* was very similar to that of *A-2*. Molecular ions of *A-6* and *A-2* lost 60 and 100 mass unit respectively corresponding to the losses of acetic and β,β-dimethylacrylic acid residues. The subsequent fragments were identical.

The NMR spectrum of *A-6* was also very similar to that of *A-2*. It had signals for 20 protons assignable as follows: six proton singlet at 8·78 τ ($(CH_3)_2C$—OH), three proton singlet at 7·84 τ (CH_3C=O), four proton multiplet around 8·34 τ (side chain CH_2—CH_2), a 1 proton multiplet at 3·94 τ (proton on carbon α to quinone ring; couples with β—CH_2 protons and quinone ring proton and is deshielded by acetylation), 2 proton singlet at 2·82 τ (aromatic protons), 1 proton doublet at 2·94 τ (*J*, 1·1 H_z) (quinone ring proton, which

appears to couple in these naphthaquinones with the protons on the carbon α to the quinone ring), one proton singlets at −2·58, −2·43 and 8·44 τ which disappear on D_2O shake and are assignable to two phenolic and one alcoholic proton respectively.

Alkaline hydrolysis yielded *A-2A*, *A-2B* and acetic acid and dehydration by tosylation followed by treatment with Na_2CO_3 yielded acetyl alkannin (*A-3*). *A-6*, therefore, has the structure V.

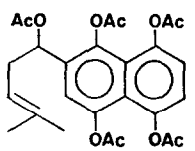


V

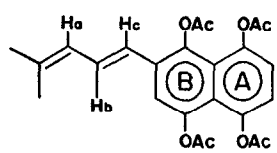
A-1

The characterization of *A-1* as alkannin β,β-dimethylacrylate was reported earlier.[1] *A-1* is the major constituent in the naphthoquinone mixture from *Arnebia nobilis* and is also the most active in inhibiting Walker carcinosarcoma in rats. Shikonin β,β-dimethylacrylate, $[\alpha]_D$ +222°, isolated earlier from *Lithospermum erythrorhizon*[5] is indistinguishable from *A-1* by TLC and IR spectra. *A-1*, however, is the levorotatory isomer: ORD (C, 0·285 mg/ml in MeOH) 223 nm (950), 250 nm (−315), 292 nm (+1020), 327 nm (−775), 400 nm (0), 500 nm (−280).

Treatment of *A-1* with acetic anhydride and fused sodium acetate in the presence of zinc dust, gave two crystalline leucoacetates *A-1B* m.p. 152° and *A-1A* m.p. 192°. *A-1B* analysed for $C_{26}H_{28}O_{10}$. Its IR spectrum with strong absorption bands at 1760, 1730, 1240 and 1190 cm^{-1} suggested the presence of both phenolic and alcoholic acetate functions in the molecule. Its NMR spectrum had a singlet at 8·02 τ for one alcoholic acetate CH_3 and signals for 4 phenolic acetate CH_3 between 7·68 and 7·72 τ. The mass spectrum of this compound had no molecular ion peak. On electron impact, the molecule loses acetic acid to give an M-60 peak at *m/e* 440. This is followed by four peaks, *m/e* 398, 356, 314 and 272 corresponding to the successive loss of 42 mass units from the four phenolic acetate residues. The base peak in the spectrum is the *m/e* 272 peak. The compound is, therefore, the penta acetate VI; replacement of the β,β-dimethylacrylyl residue by an acetyl residue having occurred during reductive acetylation by transesterification.



VI



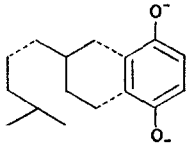
VII

Leucoacetate *A-1A* m.p. 192°, $C_{24}H_{24}O_8$ would appear to be the diene tetraacetate VII. It had λ_{max} 224 (20,330), 285 (29,540), 292 (30,160) and 324 (32,080) nm (ε) and ν_{max} 1757 and 1230 cm^{-1} (phenolic acetate). Its mass spectrum had a molecular ion peak at *m/e* 440. This ion successively loses four acetyls to give ions *m/e* 398, 356, 312 and 272 which is the

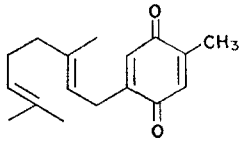
[5] I. MORIMOTO, T. KISHI, S. KEGAMI and Y. HIRATA, *Tetrahedron Letters* 4737 (1965).

most abundant ion in the spectrum. In its NMR spectrum, the four CH_3C(=O) group protons give rise to two sharp signals at 7·66 and 7·68 τ. The $(CH_3)_2C=$ doublet (J, 1·0 Hz) is at 8·06 τ. This function is, therefore, attached to a double bond as in *A-1*. Of particular interest is the downfield region of the spectrum. In addition to the signals for the three aromatic protons, there are signals for three olefinic protons. A doublet (J, 11 Hz) of two multiplets centred at 3·86 τ can be assigned to Ha as this proton should couple with Hb and the gem methyl protons. A doublet at 3·56 and 3·31 τ (J, 15 Hz) can be assigned to Hc as this is the only proton expected to give rise to a doublet. A quartet centred at 2·85 τ having a band width of 26 Hz and the lines spaced 11, 4 and 11 Hz is in agreement with Hb being associated in 11 and 15 Hz couplings. The third signal of this quartet is within the two proton signal for the ring *A* protons. This is apparent from its integration. The three protons, Ha, Hb, Hc, are, therefore, in a *trans–trans* arrangement. The unusual chemical shift of the benzylic proton (Hc) can be ascribed to it being located in the shielding cone of the acetyl carbonyl.

Although no tracer studies have been undertaken, it is likely that the alkannin molecule is derived from a potential phenol and two C_5 units joined head to tail (VIII). The isolation of 5-geranyl-2-methyl-1,4-benzoquinone (IX) from *Pyrola media*[6] provides support for this view.



VIII



IX

EXPERIMENTAL

Extraction and Separation of Naphthaquinones

Air-dried, powdered roots of *Arnebia nobilis* (2 kg) were extracted by percolation with hexane (8 × 15 l.) and solvent removed at a temp. below 50°. The dark red viscous residue obtained (50 g) was chromatographed over silica gel (1 kg). The naphthaquinones were eluted as follows:

TABLE 1

Eluant	Naphthaquinone	Weight g	TLC (R_f) Solvent System C_6H_6	TLC (R_f) Solvent System $CHCl_3$
Hexane–benzene (3:1)	*A-1*	7·5	0 80	1·0
Hexane–benzene (1:1)	*A-3*	1·5	0·60	0 93
Benzene	*A-4*	0·45	0 27	0·71
Benzene–chloroform (3:1)	*A-2*	0·95	0 08	0·51
Benzene–chloroform (3:1)	*A-5*	0 045	0·05	0·46
Benzene–chloroform (1:1)	*A-6*	0·025	0 03	0·40

[6] A. R. BURNET and R. H. THOMSON, *J. Chem. Soc.* (C), 857 (1968).

A-1

A-1 was recrystallized from hexane as dark red needles m.p. 116–117°, υ_{max} 3011, 1712, 1650, 1616, 1600, 1577, 1462, 1214, 1140 and 770 cm^{-1}, λ_{max} 223 (26,180), 280 (8740), 486 (6504) and 518 (7320) nm (ϵ). (Found: C, 68·39; H, 5·98. $C_{21}H_{22}O_6$ required: C, 68·20; H, 5·94%.)

Reductive Acetylation of A-1

Zn dust (1·0 g) was added in small portions to a refluxing solution of *A-1* (1·0 g) and fused NaOAc (1·0 g) in Ac_2O (50 ml). Refluxing was continued till the solution became a pale yellow (1·5 hr). The reaction mixture was cooled, poured into cold water (50 ml) and extracted with Et_2O. The Et_2O extractable material was chromatographed over silica gel (20 g). Elution with C_6H_6–$CHCl_3$ (3:1) and C_6H_6–$CHCl_3$ (1:1) yielded leucoacetates *A-1B* and *A-1A* respectively. *A-1A* crystallized from C_6H_6–MeOH as colourless needles m.p. 192°. (Found: C, 64·35; H, 5·09. $C_{24}H_{24}O_8$ required: C, 63·95; H, 5·45%.)

A-1B crystallized from C_6H_6–MeOH as needles m.p. 152°. (Found: C, 62·65; H, 6·15. $C_{26}H_{28}O_{10}$ required: C, 62·40; H, 5·60%.)

A-2

A-2 crystallized from hexane-benzene as dark red needles m.p. 92–94°. (Found: C, 64·47; H, 6·26. $C_{21}H_{24}O_7$ required: C, 64·94; H, 6·20%.)

Hydrolysis of A-2

A-2 (800 mg) and *N* NaOH (25 ml) were stirred at 40° for 1 hr in N_2. The reaction mixture was acidified with HCl and extracted with Et_2O (3 × 20 ml). The extract was extracted with 5% $NaHCO_3$ (2 × 10 ml), washed (H_2O), dried (anhyd. Na_2SO_4) and solvent removed. The residue was chromatographed over silica gel (10 g). Elution with hexane yielded *A-2A*, which crystallized from hexane as needles m.p. 91°. (Found: C, 66·80; H, 5·74. $C_{16}H_{16}O_5$ required: C, 66·70; H, 5·60%.) Further elution of the column with C_6H_6 yielded *A-2B*, which crystallized from $CHCl_3$–C_6H_6 as fine needles m.p. 162–164°. (Found: C, 63·12; H, 6·29. $C_{16}H_{18}O_6$ required: C, 62·74; H, 5·88%.)

Acid from A-2

The $NaHCO_3$ extract of the Et_2O soluble material obtained after hydrolysis of *A-2*, was acidified with HCl and extracted with ether. Removal of ether followed by sublimation *in vacuo* of the residue yielded colourless crystals m.p. 65–66° λ_{max} 216 (11,500) nm (ϵ). This acid was identified as β,β-dimethylacrylic acid by GLC using a column of carbowax 20M/TPA on acid-washed silanized chromosorb G (column temp. 180°). This column was found to separate β,β-dimethylacrylic, angelic and tiglic acids.

Dehydration of A-2

A solution of tosyl chloride (19 mg) in pyridine (0·3 ml) was cooled (5°) and added to a cooled (5°) solution of *A-2* (20 mg) and pyridine (0·1 ml) in C_6H_6 (1·0 ml). After 16 hr at 5°, solvent was removed under reduced pressure. 5% Na_2CO_3 (0·5 ml) and EtOH (2 ml) were added to the residue and the mixture heated at 100° for 15 min. The solution was then diluted (H_2O, 10 ml), acidified with HCl and extracted with Et_2O (3 × 7·5 ml). The extract was washed (dil. HCl and H_2O), dried (Na_2SO_4) and solvent removed. *A-1* and this product were indistinguishable by TLC.

A-5

A-5 crystallized from hexane–C_6H_6 m.p. 111–112°. (Found: C, 66·31, H, 6·55. $C_{16}H_{18}O_5$ required: C, 66·20; H, 6·20%.)

A-6

A-6 crystallized from ether–hexane, m.p. 88–90°. Hydrolysis with N NaOH in N_2 followed by working up as for *A-2* yielded *A-2A*, *A-2B* and acetic acid.

Dehydration of A-6

A-6 was dehydrated by an identical procedure to that used for dehydrating *A-2*. The product was found to be indistinguishable from alkannin acetate (*A-3*) by TLC.

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