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CYTOKININS IN THE XYLEM SAP OF BRINJAL PLANT *(SOLANUM MELONGENA L)*

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SUMMARY

Ribosyl zeatin (ZR) and zeatin (Z) were identified as the principal forms of cytokinins in the xylem sap of egg plant at flowering stage. The identification was based on their chromatographic behaviour in sephadex column chromatography and high performance liquid chromatography (HPLC). Two other cytokinins which were present in extremely low concentrations in the xylem sap showed elution patterns similar to those of isopentenyl adenine (iP) and isopentenyl adenosine (iPA) in sephadex column chromatography.

INTRODUCTION

Root tips are the important sites of cytokinin biosynthesis in plants. The cytokinins synthesised in the roots are believed to be translocated to the shoot through the xylem sap. Evidence is accumulating to show that these cytokinins translocated through the xylem sap regulate many aspects of shoot development including growth of lateral buds, flowering and senescence of leaves (Goodwin *et. al.*, 1978). A lot of interest centered round the studies aimed at isolation and identification of cytokinins from the bleeding sap/xylem sap (Skene, 1972; Hewett and Wareing, 1973; Van Staden and Dimalla, 1980). Brinjal is a tropical vegetable plant that exhibits rank vegetative growth and branching in a short period of time. Information is not available on the root produced hormones in this plant. The present study concerns the identity of cytokinins in xylem sap of brinjal plant.

MATERIALS AND METHODS

Plant material : The xylem sap was extracted from the brinjal cv. 'Arka Kusumakar' (*Solanum melongena* L.) at the flowering stage.

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Extraction of the xylem sap : The sap was collected by suction under reduced pressure from the xylem of isolated stems as described earlier (Bollard, 1960). About 1 to 1.5 ml of sap could be collected from each plant by this technique. This sap was used immediately or stored after addition of methanol in a deep freeze for subsequent use.

Ion exchange chromotography : For cation exchange column chromatography the method described by Vreman and Corse (1975) was followed using Duolite CS-101, H^+ from equilibrated at pH 10.0. The size of the column employed was 2.0 x 40 cm.

Sephadex column chromatographay : The technique described by Armstrong *et al.* (1969) was adopted employing 35 per cent or 20 per cent methanol instead of 35 percent ethanol for elution. The size of the column was 2.3 x 100 cm or 2.3 × 110 cm. The column eluates were collected in 30 or 40 ml fractions and aliquots of these equivalent to 10 ml of sap were bioassayed after solvent evaporation.

High performance liquid chromatography : A Waters Associates model 244 liquid chromatograph with two PSI pumps, a solvent programmer, an injector and an UVdetector (254nm), was employed. The column μ bondapak C_{18} (30x0.4 cm i.d) was used. The gradient elution system employed was of Kannangara *et al.* (1978) comprising of solvent (A) water: methanol (90: 10) and (B) methanol, both solvents being 0.2 M with respect to acetic acid. A linear gradient of 20 minutes run with a flow rate of 0.8 ml min^{-1} starting with 15 per cent B (in A) and ending with 42 per cent B was employed.

Bioassays : The biological activity of extracts was tested by soybean cotyledon callus test (Miller, 1965) or soybean hypocotyl test (Manos and Goldthwaite, 1976 and Newton *et al.* 1980) using the cv. Acme. The bioassays were conducted in 100 ml Erlenmeyer flasks using the basal medium described by Miller (1967) omitting kinetin when extracts were tested. For former assay 50 ml medium and for the latter 20 ml was taken in the flask. The number of explants per flask was 3 and 6 in the above two tests, respectively.

RESULTS AND DISCUSSION

In a preliminary study, 6 ml of the xylem sap was taken directly in the basal medium and tested for cytokinin activity by soybean cotyledon callus bioassay. This yielded a callus of 190 mg as against 10 mg in control. In yet another investigation, 30 ml of sap was adjusted to pH 7.6 and shaken with equal volumes of *n*-butanol. After reducing the volume *in vacuo*, both butanol soluble and butanol-insoluble fractions were dried on Whatman paper separateley and

bioassayed by soybean cotyledon callus test. It was observed that while butanol soluble fraction showed marked cytokinin activity, no activity was present in the butanol-insoluble fraction.

For detailed investigations on the nature of cytokinins in the xylem sap, from 150 ml of sap the butanol soluble fraction was separated. After evaporation of the solvent, the extract was chromatographed on a Duolite CS-101 resin (H^+ form equlibrated at pH 10.0). The column was washed with 500 ml of water (pH 10.0) and eluted with 500 ml of 1N NH_4OH in 70% ethanol. When the water eluate and NH_4OH eluate were bioassayed after solvent evaporation by soybean cotyledon callus test,cytokinin activity was detected in the NH_4OH eluate only. The NH_4OH eluate equivalent to 100ml of sap was evaporated to dryness *in vacuo*, the residue was taken up in 35 per cent methanol, filtered through a Whatman No. 1 paper and chromatographed on a sephadex LH-20 column. The column was eluted with 35 per cent methanol at a flow rate of 25 ml h^{-1}. Soybean cotyledon callus bioassay of the fractions yielded two zones of high cytokinin activity at elution volumes 470-530 ml (X_1) and 590-650 ml (X_2) and two zones of low activity at elution volumes 830-860 and 1010-1040 ml (Fig.1). The former two zones corresponded to the elution volumes of ZR and Z, respectively and the latter two to those of iPA and iP, respectively. An aliquot equivalent to 30 ml of sap, eluted at 410-710 ml from the above column consisting of factors, X_1 and X_2 was lyophilised, taken in a small quanity of 20 per cent methanol and chromatographed further on a sephadex LH-20 column using 20 per cent methanol

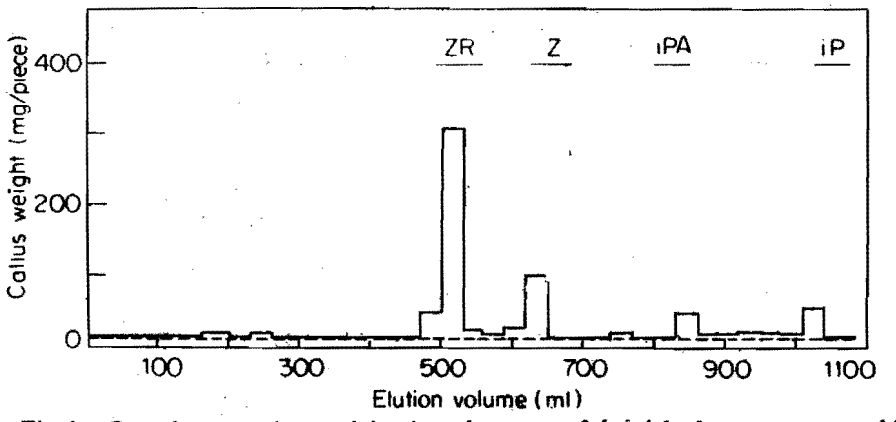

Fig. 1. Growth promoting activity in xylem sap of brinjal plant as measured by Soybean cotyledon callus test following sephadex LH-20 column chromatography using 35 per cent methanol as eluant.

as eluant. Upon this chromatography two active zones at elution volumes 520-640 ml and 760-840 ml were detected. In this system authentic samples of ZR and Z were observed to elute at elution volumes 525-605 ml and 730-810 ml, respectively (Fig. 2).

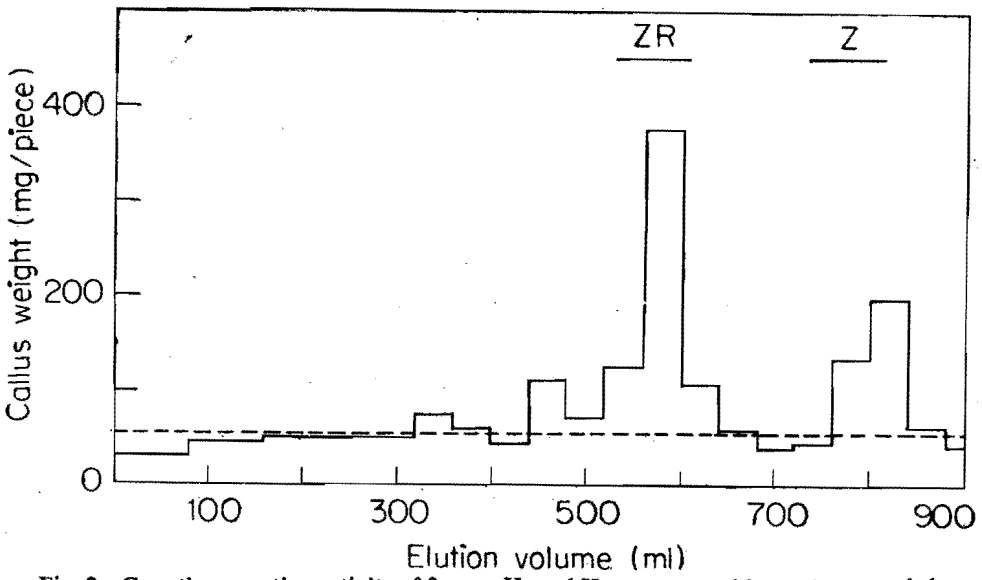

Fig. 2. Growth promoting activity of factors X_1 and X_2 as measured by soybean cotyledon callus test following sephadex LH-20 column chromatography using 20 per cent methanol as eluant.

The factors X_1 and X_2 were examined further in HPLC system separately. Authentic samples of Z, ZR, iP and iPA, and their mixture, and factors X_1 and X_2 were chromatographed separately on a μ bondapak C_{18} column using water methanol gradient system (Kannangara *et. al.*, 1978). In this system the retention times of Z,ZR, iP and iPA were found to be 7.5, 11.5, 19.5 and 25.2 min, respectively (Fig..3). The factors X_1 and X_2 yielded compounds with retention times corresponding to those of ZR and Z, respectively. Also the extracts containing these factors yielded some more UV absorbing compounds upon chromatography in HPLC system (Fig. 4 and 5). To ascertain the biological activity of the compounds, extracts containing X_1 and X_2 equivalent to 40 ml of sap were chromatographed separately in four equal runs, and for each run 5 to 7 fractions were collected. The corresponding fractions of the runs for each factor were combined, and divided into two equal lots and were bioassayed by soybean hypocotyl test after solvent evaporation. The bioassay results presented in Fig 4 and 5 show that the compounds with retention times corresponding to those of ZR and Z were biologically active in the eluates of X_1 and X_2, respectively.

The factors X_1 and X_2 may be regarded as ZR and Z, respectively. The elution patterns of thse two active compounds corresponded to those of ZR and Z in sephadex column chromatograhy when 35 per cent methanol and 20 per cent methanol were used as eluants. Also, in the HPLC system tried, these two active compounds showed retention times similar to those of the above mentioned cyto-

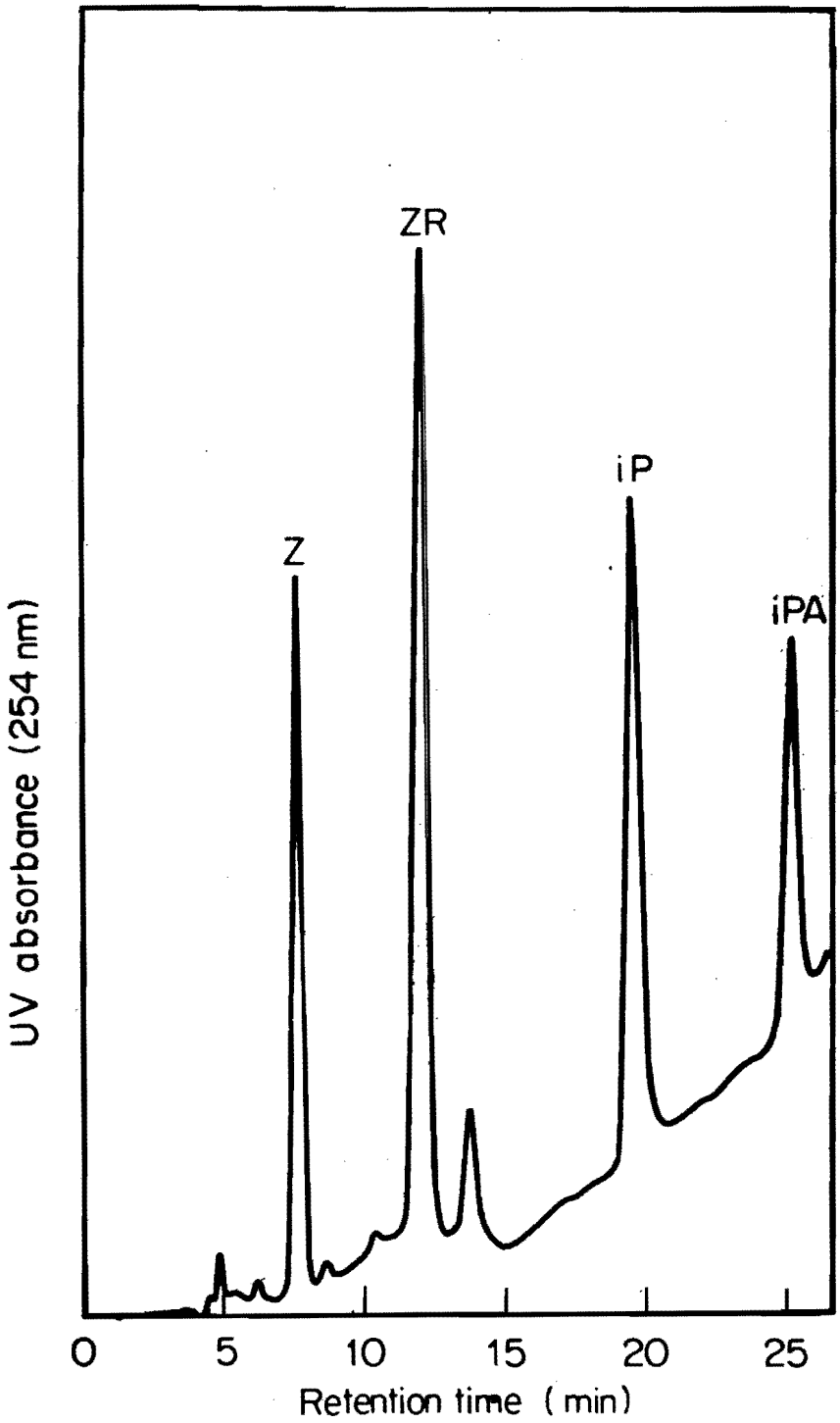

Fig. 3. Pattern of elution of zeatin, zeatin riboside, isopentenyl adenine and isopentenyl adenosine following high performance liquid chromatography of their mixture on a 30 x 0.4 cm i.d. μ bondapak C_{18} column. Solvent A was water: methanol (90 : 10) and solvent B was methanol, both solvents being 0.2M with respect to acetic acid. Elution conditions : Flow rate 0.8ml min^{-1} with 20 min gradient starting with 15 per cent B (in A) and ending with 42 per cent B.

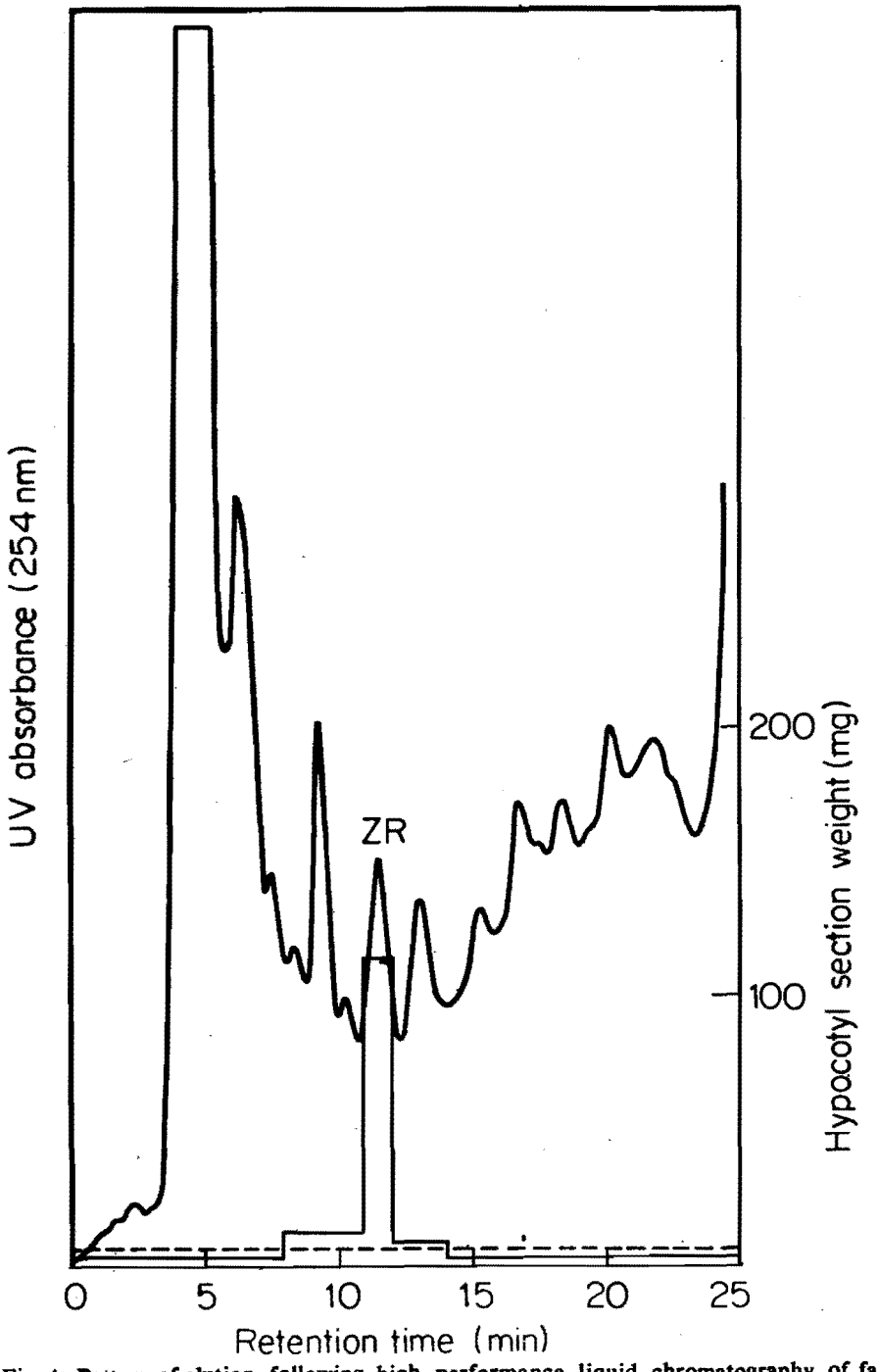

Fig. 4. Pattern of elution following high performance liquid chromatography of factor X_1 on a 30 x 0.4 cm i.d. μ bondapak C_{18} column. Biological activity as measured by soybean hypocotyl section test was superimposed over UV absorbance. The dotted line represents the hypocotyl section weight in control flasks. Solvent A was water : methanol (90 : 10) and solvent B was methanol, both solvents being 0.2M with respect to acetic acid. Elution conditions : Flow rate 0.8 ml min^{-1} with 20 min gradient starting with 15 per cent B (in A) and ending with 42 per cent B.

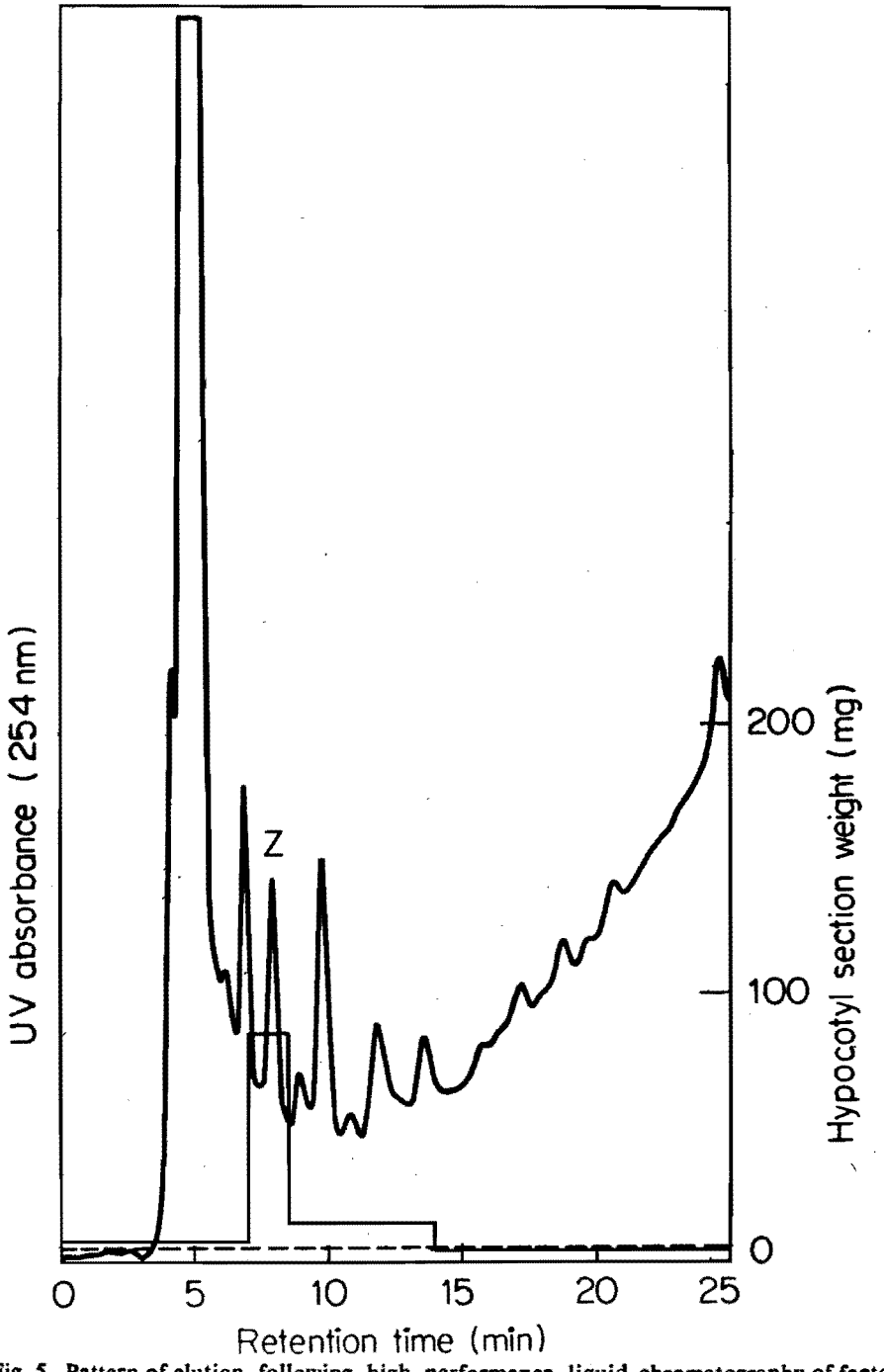

Fig. 5. Pattern of elution following high performance liquid chromatography of factor X_s on a 30 x 0.4 cm i.d. μ bondapak C_{18} column. Biological activity as measured by soybean hypocotyl section test was superimposed over UV absorbance. The dotted line represents the hypocotyl selection weight in control flasks. Solvent A was water : methanol (90 : 10) and solvent B was methanol, both solvents being 0.2M with respect to acetic acid. Elution conditions : Flow rate 0.8ml min^{-1} with 20 min gradient starting with 15 per cent B (in A) and ending with 42 per cent B.

kinins. In view of the above, ZR and Z can be considered as principal cytokinins in the xylem sap of brinjal plant. Z and/or ZR were detected in root exudates/xylem sap in other species by previous workers (Skene, 1972; Hewett and Wareing, 1973; and Van Staden and Dimalla, 1980). Though the sap contained two more cytokinin like substances, with elution patterns similar to those of iPA and iP in sephadex column chromatography, the concentrations of these were much lower than those of the above. Only recently, Van Staden and Dimalla (1980) reported the presence of iP and iPA in root sap of *Bougainvillea*.

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