It is known that with the decrease of NO concentration from 50 to 1 ppbv, the lifetime of ozone increases from 42 s to 30 min[6]. Therefore, the recycling of ozone through NO is far more efficient for Pasadena compared to WSMR. Coupling of the above fact with this experimental finding (i.e. mass-dependent fractionation with large fractionation factor) leads us to conclude that more the recycling of ozone through NO, more is the enrichment (in $\delta^{18}O$) and less is the slope from its expected value (from $O + O_2$ recombination reaction) of unity. Hence, the slope values observed in the three environments were consistent with the NO_x concentrations of these three sampling locations. Though large enrichment variations in ^{18}O were not observed consistent with their corresponding slope values, that can be interpreted as effect of other source process controlling the enrichment as described earlier. Moreover, this fractionation factor may be a useful parameter for the mathematical modelling to construct the isotopic budget of tropospheric ozone.

It was recently proposed by Bhattacharya *et al.*[13] that dissociation of ozone plays a significant role over the above temperature effect[14] in deciding the isotopic enrichment in stratospheric ozone. Moreover, the observed slope ($\Delta\delta^{17}O/\Delta\delta^{18}O$) in lower stratosphere (up to 33 km) is 0.62[4] instead of what was expected from $O + O_2$ recombination reaction (nearly unity). Additionally, it is also known that the observed stratospheric ozone concentration is half of that calculated from Chapman's cycle and is attributed to the catalytic destruction cycle. Therefore, the $NO + O_3$ reaction can be considered as a representative reaction for the catalytic cycles active in destroying ozone and hence, both the lowering of slope value as well as the increase in enrichment with altitudinal in stratospheric ozone can be accounted.

The $NO + O_3$ sink reaction follows a mass-dependent path with a significantly large associated fractionation factor ($\alpha = 1.0305$, or 30.5‰) enriching the left-over ozone. This can be used as an important parameter to model the tropospheric ozone budget. This reaction has the potential to control the relative variations of oxygen isotopic enrichments in ozone at different tropospheric environments. Additionally, this reaction can be used as a representative reaction for catalytic cycles to explain the observed slope value and the altitudinal variation of enrichment in stratospheric ozone.

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Influence of methyl jasmonate and salicylic acid in the enhancement of capsaicin production in cell suspension cultures of *Capsicum frutescens* Mill

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The cell suspension cultures of *Capsicum frutescens* were treated with two signalling compounds, salicylic acid (SA) and methyl jasmonate (MeJA), individually, and in combination. SA and MeJA were found to individually enhance capsaicin production, but when administered in combination there was no further enhancement in capsaicin production. Both the signalling compounds were also found to result in higher leaching of capsaicin into the medium. The endogenous polyamine (PA) levels were higher in the treatments with SA and lower in the treatments with MeJA. The endogenous PA levels were found to be the highest on the sixth day of culture, after which the ethylene levels were found to increase. Maximum ethylene produc-

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tion was on the 12th day of culture, coinciding with the highest capsaicin production. The correlation of capsaicin synthase levels and the increase in capsaicin production was also observed on the 12th day. It was concluded that ethylene did not show positive influence on capsaicin since, individually, SA inhibited ethylene production and MeJA enhanced it. However, both the treatments resulted in elicitation of capsaicin production.

THE role of jasmonic acid (JA) and its volatile methyl ester methyl jasmonate (MeJA) as signalling molecules in biotic and abiotic stresses[1–3] is well known, having either inhibitory or promoting effects, in the form of morphological and physiological changes[4], and also in defence metabolism[2,5]. Endogenous JA and MeJA accumulate after treatment of cell cultures with a fungal elicitor[2,5]. Treatment with jasmonates can elicit the accumulation of several classes of alkaloids[5–7], phenolics[8] or coumarins[9] in a wide variety of plant species.

Addition of MeJA to a broad range of plant-cell suspensions resulted in higher accumulation or *de novo* synthesis of secondary metabolites[5]. Jasmonates are expected to be the chemical signal compounds in the process of elicitation leading to *de novo* gene transcription, and finally, the biosynthesis of natural products in cultured plant cells[10].

The elicitation of capsaicin production under the influence of microbial elicitors has been studied[11]. *Capsicum* contains flavonoids present as both flavonol and flavonone glucosidic conjugates[12]. Flavonoids and phenolic compounds are important dietary antioxidants[13,14] and along with vitamins, they play an important role in cancer chemoprevention[15]. Capsaicin present in the oleoresin imparts pungency. Continuous production of capsaicin is possible using column reactors, employing immobilized cells[16]. In this context, it was of interest to study the elicitation of capsaicin under the influence of MeJA and salicylic acid (SA) in cultured cells of *Capsicum frutescens*. The capsaicin biosynthetic pathway has two distinct branches. One utilizes phenylalanine and gives rise to the aromatic component, vanillylamine via the phenylpropanoid pathway[17,18]. The second forms the branched-chain fatty acids by elongation of deaminated valine. The first part of the reaction sequence comprising the aromatic pathway is shared with other pathways of general phenylpropanoid metabolism and is common to all higher plants. The latter part of the reaction sequence from ferulic acid through vanillin and vanillylamine to capsaicin is found only in the fruits and cell cultures of *Capsicum*[12]. Capsaicin is the result of the condensation of 8-methyl-6-nonanoic acid with vanillylamine by capsaicinoid synthase. The activity of the terminal enzyme of the capsaicin biosynthetic pathway, capsaicin synthase (CS) was also studied under these treatments. Stress has been shown to promote ethylene production in plant tissues[19]. SA and MeJA are known to have counteracting effects on ethylene production. Hence, it was of interest to study the ethylene and polyamine (PA) levels, as they are known to affect the biosynthesis of each other. Thus, in this investigation, the interrelationship of elicitor-mediated capsaicin production with ethylene and PAs has been studied.

Seeds of *C. frutescens* Mill. (Arch 226), high-pungent variety were obtained from Ankur Seeds, Nagpur, India. The callus was initiated from seedling of *C. frutescens* on MS medium[20] containing 9.05 μM 2,4-D and 2.32 μM kinetin. The cultures were incubated at 25 ± 2°C under cool light (4.4117 Jm^{-2} s^{-1} 18 h day^{-1}). The callus of *C. frutescens* was maintained by regular subculturing at three-week intervals. The callus tissue of *C. frutescens* (approximately 1 g) was transferred to 40 ml of liquid MS medium of the same hormonal combination, but without agar in 150 ml Erlenmeyer flasks. It was incubated on a rotary shaker (New Brunswick Scientific Co, Inc, New Jersey) at 90 rpm in light as given above. The fine suspension was selectively subcultured removing the clumps to obtain a uniform suspension culture. This suspension culture was maintained by periodic subculturing at two-week intervals.

MeJA was obtained from Aldrich Co, USA. A known volume of MeJA was diluted with ethanol and filter-sterilized before addition to the culture medium. SA was obtained as its sodium salt from Sigma Co, USA. It was added to the medium prior to autoclaving. Media constituents were obtained from Hi-Media, Bombay.

Growth of the cell suspension was measured in terms of fresh weight. The culture was filtered through a nylon mesh and the cells were placed between the folds of blotting paper to remove excess moisture, and then the fresh weight was taken.

For the extraction of capsaicin from suspension cultures, one gram of cells was ground well with neutralized glass powder (100 mg) using a mortar and pestle, and extracted thrice with 25 ml of ethyl acetate. The extract was centrifuged at 2000 rpm for 15 min and the supernatant was evaporated. The residue was then dissolved in known aliquots of ethyl acetate and used for capsaicin analysis.

Capsaicin from the culture media was extracted thrice with 20 ml of ethyl acetate, each time in a separating funnel. The ethyl acetate layers were pooled and evaporated. The residue was dissolved in known volume of ethyl acetate for analysis[11].

The capsaicin extracted from the callus and media was estimated by high performance liquid chromatography (HPLC; Shimadzu LC 10 A) following the method of Hoffman *et al.*[21]. The quantification of capsaicin was done on a C18 Bondapak column with detection at 280 nm. The isocratic mobile phase was acetonitrile : water (1% acetic acid) (40 : 60 v/v) and a flow rate of 1 ml/min was used.

The assay of capsaicin synthase from the callus of *C. frutescens* was done following the method of Iwai *et al.*[22]. The callus was extracted with 0.1 M potassium

phosphate buffer, pH 6.8 with 5 mM 2-mercaptoethanol. The extract was centrifuged at 10,000 rpm for 40 min and the supernatant was used as the crude enzyme extract. The assay mixture contained 0.5 mM potassium phosphate buffer (pH 6.8, 40 µmol), magnesium chloride (1 µmol), ATP (1 µmol), vanillylamine hydrochloride (5 µmol), nonanoic acid (5 µmol) and the enzyme extract (300 µl) in a final volume of 0.5 ml. The assay was carried out at 37°C for 2 h, and the reaction was terminated by the addition of 0.3 ml of 98% ethanol. Capsaicin was extracted with ethyl acetate (10 ml) and estimated by HPLC. One unit of enzyme activity was defined as 1 µM of capsaicin formed per mg protein in one hour. The protein was determined by the method of Lowry *et al.*[23].

Extraction of the endogenous PAs in callus cultures was carried out by acid hydrolysis of perchloric acid (PCA)-soluble and PCA-insoluble extracts[24]. The callus was extracted in 5% cold PCA at a ratio of about 100 mg/ml PCA. After extraction for 1 h in an ice bath, the samples were centrifuged at 10,000 rpm for 20 min and the supernatant containing the free PAs and the pellet containing the conjugated PAs were benzoylated according to the method of Redmond and Tseng[25]. The benzoylated PAs were analysed by HPLC, as reported earlier[26]. Each sample was replicated thrice for HPLC and an average of the three values was expressed as µg/g fresh weight of the tissue.

Ethylene release from the callus cultures of *C. frutescens* was measured under various treatments. The callus was inoculated in serum-stoppered bottles for the estimation of ethylene by gas chromatography (GC-15A, Shimadzu Ltd, Japan)[27] as reported earlier by Bais *et al.*[28].

The fresh weight, capsaicin content and CS activity were estimated in five samples and the average was calculated. For the PA content and ethylene levels, an average of three replicates was calculated, and the standard error was calculated following the method of Freund and Perles[29].

MeJA and SA were found to individually influence the production of capsaicin in cell-suspension cultures of *C. frutescens*. Hence, the effect of the two in combination was studied to understand the interaction between the different elicitors.

The maximum biomass accumulation in the cell-suspension cultures of *C. frutescens* was observed on the 15th day of culture. When the cell-suspension cultures of *C. frutescens* were treated with 200 µM level of SA, biomass accumulation was slightly higher (13.1 ± 0.6 g/culture) compared to the control (11.39 ± 0.5 g/culture). MeJA was not found to affect the growth of the culture at the 0.05 µM level (11.25 ± 0.5 g/culture). Treatment with a combination of SA and MeJA reduced the growth of the cells (Figure 1 *a*).

The total capsaicin levels were the highest on the 12th day of culture, which correlated with higher activity of CS. After the 12th day, the activity of CS and the levels of capsaicin were found to be lower. The treatment wherein 200 µM of SA was administered to the culture, resulted in the higher level of total capsaicin (46.64 ± 2.3 µg/culture) compared to the control (29.25 ± 1.4 µg/culture) on the 12th day of culture (Figure 1 *d*). This correlated with higher activity of CS on the same day in 200 µM-treated cultures (6.9 ± 0.34 units). The total capsaicin content was higher in cultures treated with 0.05 µM of MeJA (40.19 ± 2 µg/culture; Figure 1 *d*). When 200 µM of SA was added to the culture medium, it was observed that there was a 1.5-fold enhancement in the capsaicin content in the cells compared to the control, on the 12th day of culture (Figure 1 *b*). Treatment of the cells with SA and MeJA resulted in maximum release of capsaicin into the medium on the 15th day (24.16 ± 1.2 µg/40 ml media). Administration of SA in combination with MeJA resulted in marginal increase in total capsaicin content. The treatment wherein SA was administered in combination with MeJA also resulted in higher CS activity (6.2 ± 0.3 units; Figure 1 *e*).

The endogenous titres of PA were higher in the treatment where 200 µM of SA was added to the medium (869 ± 43; 436 ± 21; 442 ± 22 µg/g fresh wt) compared to the control (745 ± 37; 413 ± 20; 322 ± 16 µg/g fresh wt) on the sixth day of culture. Administration of MeJA resulted in lower levels of PA. The addition of MeJA along with SA did not further enhance the endogenous titres of PA (Figure 2). The higher levels of PA in treatments with SA could be growth-related.

The highest levels of ethylene were observed on the 12th day of culture, which coincided with the maximum capsaicin accumulation. The levels of ethylene were low in treatment with SA (83.98 ± 4.19 µmol/ml) compared to the control (102.56 ± 5.12 µmol/ml) on the 12th day of culture. Treatment of the cells with MeJA resulted in higher levels of ethylene (128.28 ± 6.41 µmol/ml). When SA was administered along with MeJA, the levels of ethylene were comparable to the control (Figure 3).

SA is known to inhibit ethylene biosynthesis. In pear cell-suspension cultures, it reduced the conversion of aminocyclopropane-carboxylic acid (ACC) to ethylene[30]. Saniewski and Wegrzynowicz-Lesiak[31] showed that MeJA stimulated ethylene evolution and ACC oxidase activity during gum induction in stems of tulips. MeJA was also reported to promote anthocyanin accumulation and ethylene production in uncooled and cooled tulip bulbs[32].

Some elicitor compounds actually antagonize each other: Vidal *et al.*[33] observed cross-interference between two distinct pathways for induction of plant-defence genes *Erwinia* elicitors and SA. Schweizer *et al.*[34] also reported the additive and negative interactions between JA and an elicitor to the existence of separate, antagonistic, signalling pathways. Pre-incubation of parsley suspension cells with MeJA greatly enhanced subsequent induction by an elicitor preparation[35]. However, conditioning of Ohelo cells with either MeJA or elicitors before addition of an-

other compound did not enhance anthocyanin accumulation, because higher anthocyanin levels were produced in the treatment with MeJA applied alone[36]. The diversity of the plant jasmonate-like signalling system[37] in different plant species could result in the different responses to conditioning. In rice, diethyldithiocarbamic acid, an inhibitor of jasmonate biosynthesis, which enhanced JA level in treated leaves, acted indirectly to enhance JA via stress-related signals from cells affected by phytotoxic effects[34].

Thus in *C. frutescens* cell-suspension cultures, SA and MeJA were found to individually enhance the capsaicin production, but the administration of both these signalling compounds together did not result in further enhancement of capsaicin production. PA levels were observed to be the highest on the sixth day of culture (Figure 2) after

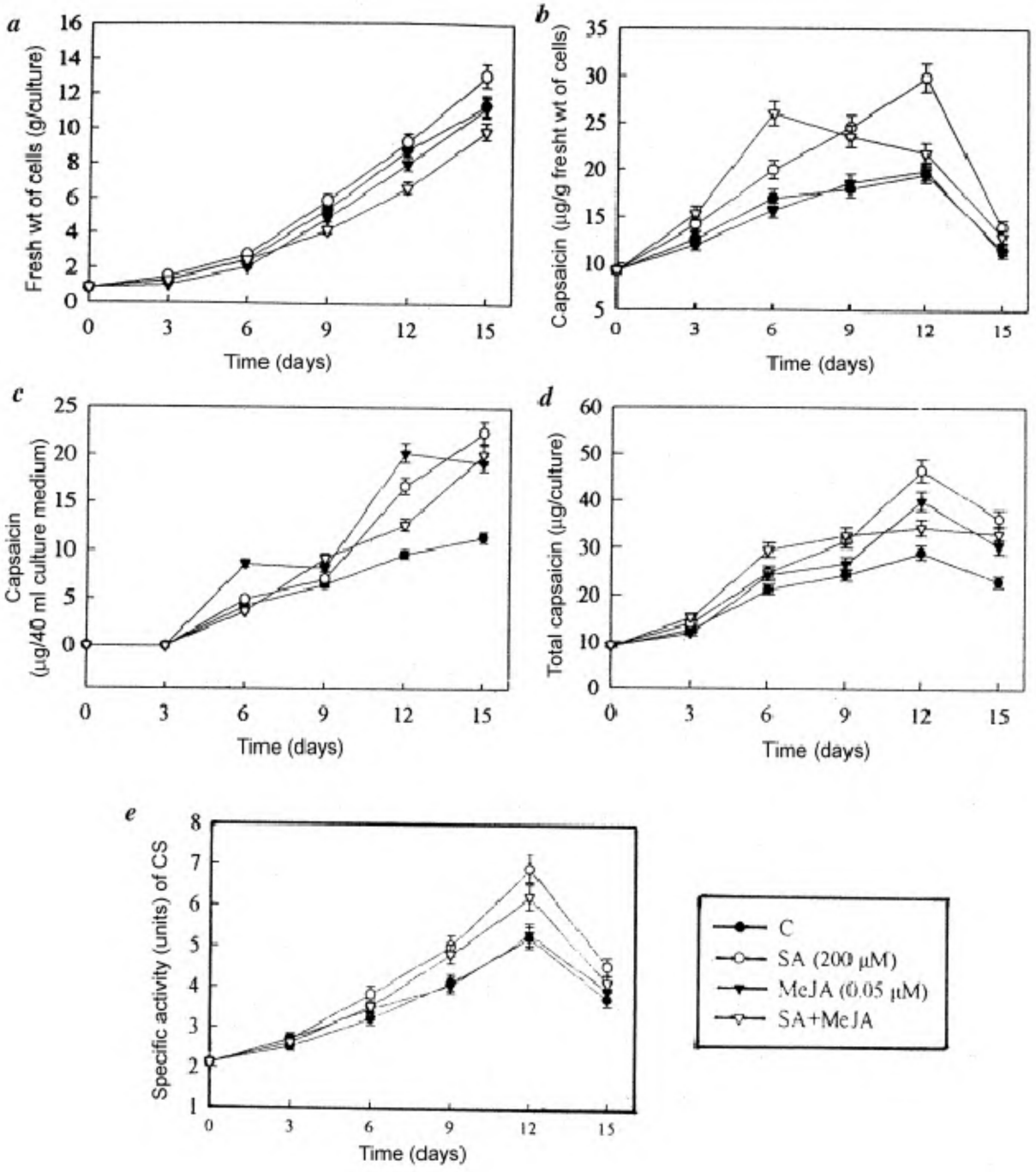

Figure 1. Effect of salicylic acid (SA), elicitor and methyl jasmonate (MeJA) on (***a***) growth, (***b***) capsaicin content in cells, (***c***) capsaicin content in media, (***d***) total capsaicin content and (***e***) specific activity of capsaicin synthase (CS) in cell-suspension cultures of *C. frutescens*.

which it decreased. Ethylene levels were found to increase after the ninth day, with a maximum accumulation on the 12th day coinciding with the highest capsaicin production. This could suggest the utilization of SAM for ethylene production after the 6th day of culture. But ethylene levels did not appear to have a major role in capsaicin production, as SA inhibited ethylene production and MeJA resulted in higher levels of ethylene (Figure 3), but both these treatments resulted in higher levels of capsaicin. Increased production of capsaicin will be useful in cell-culture process for enhancement of the yield.

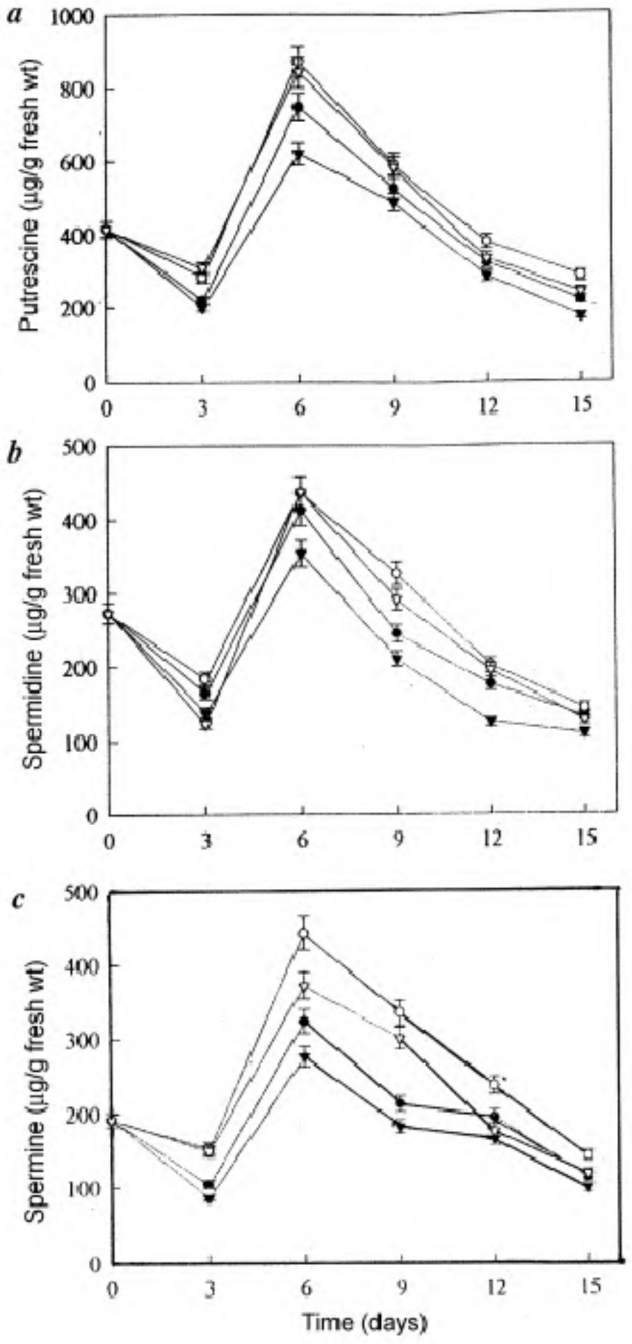

Figure 2. Effect of salicylic acid and methyl jasmonate on levels of total PA in cell-suspension cultures of *C. frutescens*. —●—, C; —○—, SA (200 µM); —▼—, MeJA (0.05 µM); —▽—, SA + MeJA.

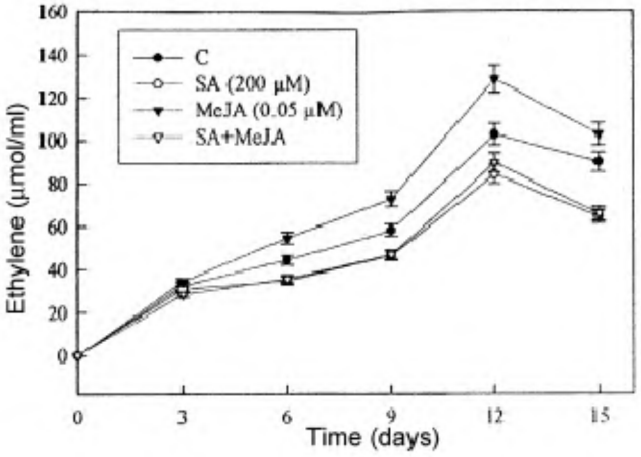

Figure 3. Effect of salicylic acid and methyl jasmonate on levels of ethylene in cell-suspension cultures of *C. frutescens*.

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Water-mediated supramolecular β-sheet from a short synthetic peptide containing non-coded amino acids

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The crystal structure of a synthetic, terminally blocked tetrapeptide *t*-Boc-β-Ala-L-Leu-Aib-L-Val-OMe reveals that the peptide adopts an overall extended backbone conformation. It self-assembles in the solid state to form an intermolecularly hydrogen-bonded β-sheet-like structure mediated by water molecules.

β-ALANINE (β-Ala) is the first member of ω-amino acids, which has been recently implicated in *de novo* peptide and protein design. The presence of additional polymethylene spacers in these amino acids, between N and C^{α} atoms, provides increased flexibility to the peptide backbone and allows us to design and characterize several diverse structures[1–3]. Many crystal and solution structures of β-Ala and γ-aminobutyric acid (γ-Abu) in both linear and cyclic peptides, reveal a variety of supramolecular helices and β-sheet structures[4,5]. The supramolecular architecture has numerous applications in material and biological sciences[6], e.g. the design and synthesis of model peptides, which form supramolecular β-sheets in crystals and amyloid-like fibrils in the solid state, is one of the convenient approaches to elucidate and understand the fibrillogenesis process at the atomic level. Previously, it has been demonstrated that synthetic peptides containing alkyl spacers (β-alanyl residues) form supramolecular β-sheets and they further self-assemble into amyloid-like fibrils[4,5]. As a continuation of our research in designing and constructing a unique supramolecular peptide architecture, we report the investigations on conformational analysis of a tetrapeptide, *t*-Boc-β-Ala-L-Leu-Aib-L-Val-OMe (Figure 1).

The title peptide has been synthesized by conventional solution phase methodology[7]. Single crystals, obtained from methanol and water mixtures (in 1 : 1 ratio), were monoclinic. Intensity data were recorded with CuK_{α} radiation on an Enraf–Nonius CAD-4 diffractometer[8] using variable scan speed ($\Delta\omega = 0.80° + 0.14° \tan\theta$), with $\omega/2\theta$ scan in bisecting geometry mode. Crystals were stable during data collection. Data intensity and background counts were taken in the ratio 2 : 1. A total of 3823 intensities were measured and corrected only for Lorentz and polarization effects. Reduced data had merged *R*-factors: $R_{int} = 0.021$ and $R_{\sigma} = 0.015$. Applying direct-phase determination technique in SHELX 97 (ref. 9) 80 phase-sets were generated from 352 largest E-values above 1.2. There were two solutions having the least value of combined figure-of-merit. *E*-maps were computed for both the sets, and the one which gave interpretable results had a molecular fragment containing 24 non-hydrogen atoms. Using this as a model, difference Fourier maps were generated by least-square refinement carried on F^2. The remaining atoms, including one water molecule, were located from successive Fourier maps. Polar axis restraints in the space group C2 were applied using the method of Flack and Schwarzenbach[10]. Hydrogen atoms, attached to the backbone and side chain atoms, were geometrically idealized and were assigned isotropic displacement parameters, 20% more than the atoms to which they are bonded (25% in case of methyl groups). Hydrogen atom positions of water molecule were not

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