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# Do Tomato Plants Contain Endogenous Indoleacetylaspartic Acid?

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**Abstract.** The data of Row *et al.* (1961) on endogenous indoleacetylaspartic acid in tomato plants were reexamined. It was shown that two indole compounds were present in ethanolic extract of tomato stems and leaves. The predominating substance was concluded to be N-malonyltryptophan on account of its UV- and IR-spectra, colour reactions with the Ehrlich and Salkovski reagents and products of acidic and alkaline hydrolyses. The chemical nature of the second indole compound remained unknown. It differed from N-malonyltryptophan, N-acetyltryptophan, indoleacetylaspartic acid and indoleacetic acid and may result from non-enzymatic transformation of N-malonyltryptophan.

The data on the presence of endogenous IAAsp in plants are important for answering the question if IAAsp synthesis and degradation are the means for the regulation of endogenous IAA content. Endogenous conjugates of IAA, 4-Cl-IAA, benzoic and phenylacetic acids with aspartic acid were distinctly observed in maturating seeds (KLAMBT 1960, HATTORY and MARUMO 1972, GIANFAGNA and DAVIES 1980, COHEN 1981). The evidence of endogenous IAAsp in vegetative plant organs is convincing because the difference between the compounds found in them and N-malonyl-tryptophan was not sufficiently proved (ROW *et al.* 1961, OLNEY 1968, WILLIAMS and

TABLE 1
Comparison of Rf values of IAAsp and the indole compound isolated from tomato

| Solvent | IAAsp | Indole compound |
|---|---|---|
| Chloroform: ethyl acetate: HCOOH (5 : 4 : 1) | 0.32 | 0.35 |
| 2-Propanol: ammonia: $H_2O$ (9 : 7 : 4) | 0.1 | 0.13 |
| 2-Propanol: $H_2O$ (4 : 1) | 0.85 | 0.85 |

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STAHLY 1970). In some papers the presence of IAAsp was evidenced by the biotest with *Avena* coleoptile sections (TILLBERG 1974, SALEH and HEMBERG 1980) which made this identification unreliable. The publication of ROW *et al.* (1961) is often cited as providing evidence for naturally-occurring IAAsp in tomato plants. Nevertheless, the presence of MT was shown to be possible in tomato (GOOD and ANDREAE 1957, SCHNEIDER *et al.* 1972). In this paper the data on endogenous indole compounds isolated from tomato are presented.

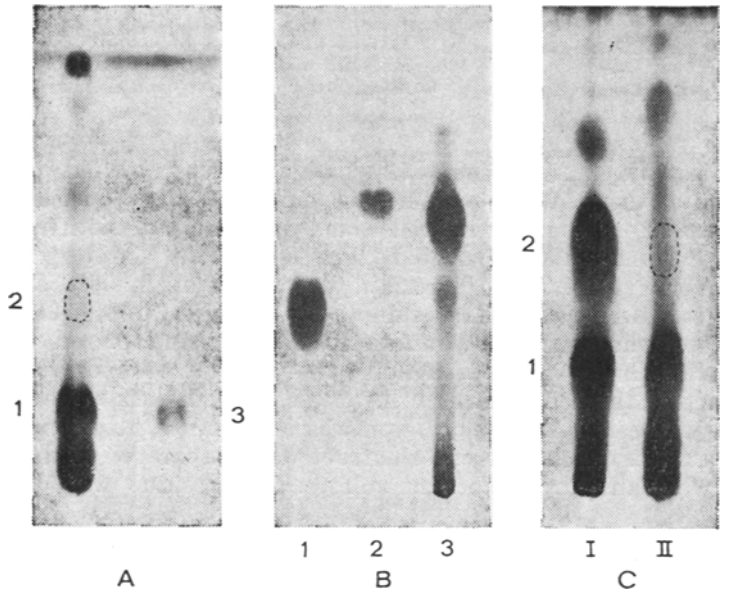


Fig. 1. A — The chromatogram of the extract of tomato stems and leaves, 1 and 2 — Ehrlich-positive spots of tomato extract, 3 — IAAsp. B — The effect of heating on the tomato indole compounds. 1 — Tomato indole compound (supposedly N-malonyltryptophan), not heated 2 — N-acetyltryptophan; 3 — the same tomato indole compound heated at 145 °C for 45 min then dissolved in ethanol and applied on the chromatogram. C — The chromatogram of the extracts of tomato stems and leaves alfter 4 month' storage (I) and just after isolation (II). 1 and 2 — as on A. — In all cases the extracts were spotted on SILUFOL UV 254 plates and developed in chloroform : ethyl acetate : HCOOH (5 : 4 : 0.5 for A and C, 5 : 4 : 1 for B, v/v). Purification steps during isolation: C (I, II) — crude tomato extracts; A (— the same tomato extract after DEAE-cellulose; B (I) — MT after DEAE-cellulose and TLC.

## MATERIAL AND METHODS

Tomato plants (*Lycopersicon esculentum* cv. Peremoga 165) were grown in a greenhouse for 5—6 weeks. Stems and leaves (*ca.* 3 kg) were frozen by liquid nitrogen and extracted by 7 l of 80% ethanol for 24 h at 10 °C. The extraction with 4 l of 80% ethanol was repeated twice at a room temperature. This procedure took 3 h. Ethanol was removed from the extract under reduced pressure at 30 °C and aqueous residue was adjusted to pH 9 with

*Abbreviations used:* MT = N-malonyl-d-tryptophan; IAAsp = indoleacetylaspartic acid; IAA = indoleacetic acid.

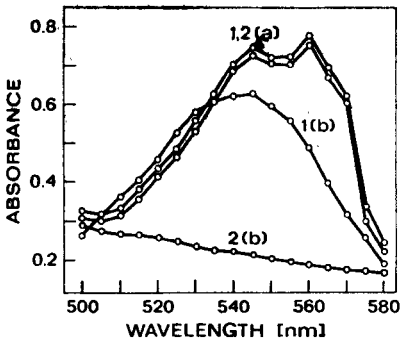


Fig. 2. The spectra of coloured products of the reactions of IAAsp (1) and tomato indole compound (2) with the Ehrlich (a) and Salkovski (b) reagents.

sodium bicarbonate. After removal of pigments by 10-fold partitioning against diethyl ether aqueous residue was acidified to pH 2.5 with 6N $H_3PO_4$ and extracted 12—15 times with redistilled peroxide-free diethyl ether. The acid ether extract was dehydrated with anhydrous sodium sulfate and evaporated to dryness. The dry residue was taken up in 96% ethanol and applied on a DEAE-cellulose column ($OH^-$ — form, 15× 200 mm). The column was washed with 200 ml distilled water and eluted with 0.1 M $KH_2PO_4$ pH 3. The fractions with indole compounds were reduced in volume and extracted with diethyl ether which was then shaken three times with 2% $NaHCO_3$. The bicarbonate fraction was acidified to pH 2 and extracted with ether 10—12 times. The ether extract was evaporated to dryness after treatment with anhydrous sodium sulfate. The residue was dissolved in

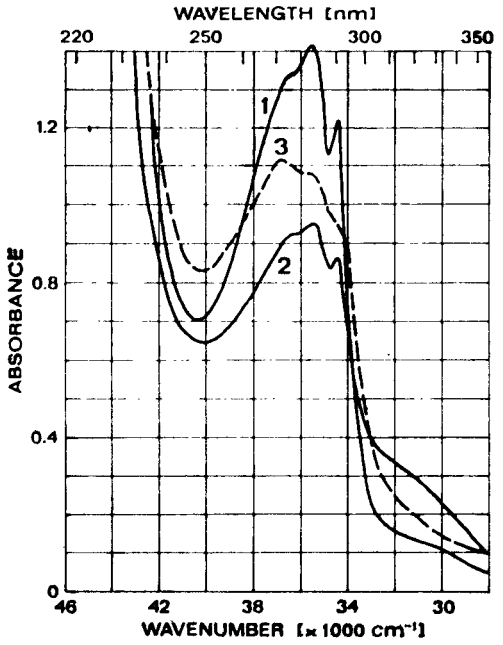


Fig 3 UV — spectra of MT from soybean cells (1), tomato indole compound (2) and IAAsp (3).

96% ethanol and used for preparative TLC (1 mm thick layer of Silicagel L 5/40 Chemapol, ČSSR) in chloroform — ethyl acetate — formic acid (5 : 4 : 1). The zones corresponding to indole compounds (verified by UV fluorescence) were scraped off the plates and eluted with 80% ethanol. Samples of this eluate were used for hydrolysis in 6 M HCl or in 1 M KOH (KOZARENKO 1975) and for colour reactions with the Ehrlich and Salkovski reagents. The

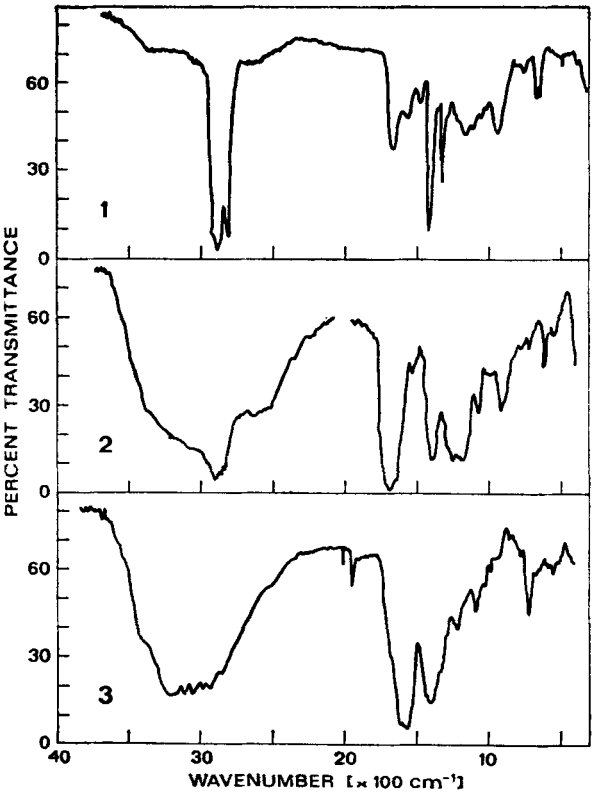


Fig. 4. IR—spectra of IAAsp (1), tomato indole compound (2) and MT from soybean cells (3).

determination of the nitrogen compounds in hydrolysates was made by an automatic amino acid analyzer (Microtechna, ČSSR). Malonic acid was determined in alkaline hydrolysate by the method of BENTLEY (1952). UV-spectra were made in a spectrophotometer Specord UV VIS (Carl Zeiss, Jena, GDR) and IR-spectra in Specord 75-IR (Carl Zeiss, Jena, GDR).

Di(cyclohexylammonium) $\cdot H_2O$ salt of IAAsp (Calbiochem) and N-malonyl-d-tryptophan obtained by biotransformation of d-tryptophan in soybean cell culture were used as standards. The experiments were repeated 4 times.

## RESULTS AND DISCUSSION

Fig. 1A shows two Ehrlich-positive compounds in an acid ether fraction of ethanolic extract of tomato stems and leaves. The most prevailing compound was similar to IAAsp by Rf on chromatograms and colour reaction

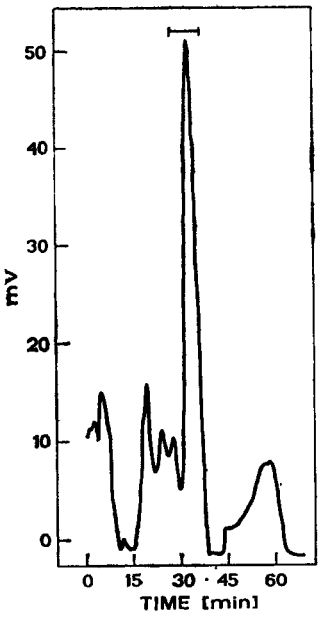


Fig. 5. Amino acid analysis of alkaline hydrolysate of tomato indole compound. Horizontal bar corresponds to the retention time of tryptophan.

with the Ehrlich reagent (Table 1). But colour reaction of this compound with the Salkovski reagent differed from this of IAAsp. This was more evident on the spectra of coloured solutions. As shown in Fig. 2, the spectra of IAAsp and tomato indole compound after treatment with the Ehrlich reagent were identical while those with the Salkovski reagent were different: IAAsp had a maximum at 545 nm but tomato indole compound had no maxima at 500—580 nm.

UV-spectra of tomato indole compound and MT had maxima at 274, 281 and 291 nm and UV-spectrum of IAAsp — at 273 and 281 nm (Fig. 3). The absence of purple colour with the Salkovski reagent and three maxima in UV-spectrum may be considered as an indication of the presence of tryptophan moiety in tomato indole compound.

Fig. 4 shows that IR-spectra of tomato indole compound and MT had much more in common compared with IR-spectrum of IAAsp.

Only $NH_3$ was found in acid hydrolysate of tomato indole compound, while hydrolysis of IAAsp usually gives aspartic acid. Instability in an acid solution is known to be also characteristic of tryptophan and its derivatives. Tryptophan only and no other amino acids were found after alkaline hydrolysis of tomato indole compound (Fig. 5). In acid ether extract of this hydrolysate one organic acid was found on TLC after spraying with bromocresol green (Fig. 6). The Rf of this acid coincided with the standard of malonic acid. Alkaline hydrolysis of IAAsp usually produced aspartic acid and IAA. Heating of tomato indole compound at 145 °C for 45 min caused its transformation to substance having the same Rf on chromatograms, as N-acetyltryptophan (Fig. 1B) appeared from N-malonyltryptophan as a result of such treatment (GOOD and ANDREAE 1957).

Thus, all data received evidenced unequivocally that prevailing indole substance in tomato plants was MT but not IAAsp. The content of MT was

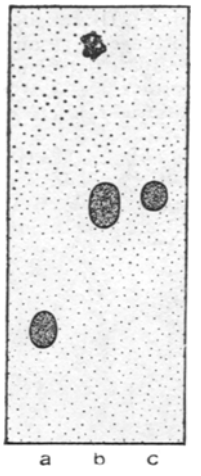


Fig. 6. TLC analysis of alkaline hydrolysate of tomato indole compound. a — Tomato indole compound before hydrolysis, b — acid ether extract of alkaline hydrolysate, c — malonic acid (standard). SILUFOL UV 254, solvent — chloroform : ethylacetate : HCOOH (5 : 4 : 0.5) sprayed with bromocresol green.

shown to be over a range of 30—60 mg per 1 kg fresh matter (the losses during purification and extraction remained unknown). The results indicate that a large amount of indoles is stored in tomato plants in the form of MT.

We observed that MT content in a crude ethanolic extract decreased gradually during 3—4 month' storage in a refrigerator, whereas the content of the second less polar indole substance increased (Fig. 1C). The nature of this substance remains unknown but according to its chromatographic properties it is not N-acetyltryptophan, MT, IAAsp or IAA. An increase of the content of this substance during storage at the expense of MT does not allow to consider it as a naturally-occurring indole compound of tomato since some amount of it can be formed during extraction and purification procedures.

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## BOOK REVIEW

MOKRONOSOV, A. T.: ONTOGENETICHESKIĬ ASPEKT FOTOSINTEZA. [ONTOGENETIC ASPECT OF PHOTOSYNTHESIS.] — Nauka, Moskva 1981. 196 pp. 2.30 Rbl.

The book is the first publication in the recent Soviet literature devoted to the ontogenetic aspect in plant photosynthesis. The author's aim was to analyse individual components of the photosynthetic apparatus and their patterns of changes during leaf and plant ontogeny and to evaluate how they contribute to the ontogenetic changes of the complex phenomenon of photosynthesis.

The book starts with changes in mesostructure during leaf development, summarizing ontogenetic events in the leaf area, cell numbers and sizes, numbers and volumes of chloroplasts, *etc.* On several examples (*Solanum tuberosum*, *Phaseolus vulgaris*, *Xanthium italicum*, *Nicotiana rustica*, *Acer platanoides*, *Hordeum vulgare*) the patterns in leaf photosynthetic rate from leaf unfolding to leaf senescence are related to variations in mesostructure during leaf ontogeny. The following Chapter entitled "Ontogenetic and cytogenetic factors of photosynthesis" brings an overview of endogenous factors which may influence developmental changes in photosynthesis. The dynamics of photosynthesis and photorespiration during leaf ontogeny is confronted with the changes in conductances for $CO_2$ transfer, chlorophyll content, photosystems ratio, assimilation number and photosynthetic enzyme activities. Special interest is turned to the effects of polyploidy, heterosis, photoperiodism and phenotype on the photosynthetic performance of plants. The photosynthesis of wild type plants and agricultural plants, and the interrelationships between photosynthetic rate, translocation of photosynthates and plant productivity are compared. In the Chapter "Developmental peculiarities of the photosynthetic carbon metabolism" the different types of $CO_2$ fixation ($C_3$, $C_4$, CAM), carbon metabolism in periods of high $CO_2$ concentration and the heterotrophic (dark) $CO_2$ fixation are treated. In the last Chapter "Endogenous control of photosynthesis in the whole plant" the source—sink hypothesis, the photosynthate concentration as a factor controlling photosynthesis, and the role of growth regulators and inhibitors are evaluated. The book contains a summary in Russian, a list of 473 references but no author, subject or plant indexes are added.

The book summarizes our up-to-date knowledge of photosynthetic patterns during leaf and plant ontogeny based on literature data and on the broad experimental experience of the author. Fields where further research is needed are stressed, and some new aspects of plant physiological studies in connection with photosynthesis are detected. It is a pity that no summary in English was added; the translation of the book is highly desirable. The book is recommended to specialists in plant physiology, namely in physiology and biochemistry of photosynthesis, and to post-graduate students, too.

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