7. A. A. Bothner-By, Adv. Magn. Reson. Spectrosc., 1, 195 (1965).
8. H. Booth, Prog. Nucl. Magn. Reson. Spectrosc., 5, 212 (1968).
9. E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, Conformational Analysis, Interscience, New York (1965) [Russian translation, Mir, Moscow (1969), p. 254].

# AMOUNT OF STEROLS AND TRITERPENOIDS IN LEAVES AND PETIOLES OF LEAF-SHEDDING AND STANDARD LINES OF COTTON PLANT

Ya. V. Rashkes, A. M. Rashkes,
M. M. Kiktev, N. K. Khidyrova,
Kh. M. Shakhidoyatov,
and A. A. Abdullaev

UDC 547.92+633.511+543.51

The amount of free and bound sterols and triterpenoids in the leaf blades and petioles of the variety Tashkent-1 and the deciduous lines L-275 and L-470 in the budding-flowering, maturing, and leaf-fall phases have been compared. In the leaf blades, the maximum accumulation of free sterols in the deciduous lines is observed in an earlier phase than in the variety Tashkent-1, and this is most characteristic for sterols saturated in the C-17 side chain. The dynamics of the change in the content of unsaturated sterols is individual for each line. The nature of the change in the amount of free sterols in the petioles is typical for each line and does not depend on the nature of the sterol for the variety Tashkent-1 this index falls sharply in the leaf-fall phase while for L-275 the process is retarded between the second and third phases, and for L-470 in the same period the amount of these substances rises sharply. In the leaf blades, as a rule, the level of sterol esters changes in parallel with the levels of free sterols, and in the petioles in the antiparallel direction between the first and second phases. In all the samples free and bound amyrin was detected. The dynamics of the change in the amounts of these substances in the three lines were different.

One of the alternatives in the solution of the problem of pre-harvesting defoliation of the cotton plant is the creation of selection lines with earlier times of lead-fall. Since mutant genes affect the biosynthesis of secondary metabolites [1], there is a definite point in studying the qualitative and quantitative composition of these compounds in lines possessing a known genetic characteristic and comparing it with the corresponding characteristic of lines (varieties) of cotton plant that have not been subjected to mutations. One of the results of the comparison may be the creation of methods for acting on industrial varieties with nontraditional ecologically pure substances the purpose of which consists in changing the balance of lead-fall regulators in the required direction.

Searches for a natural "aging factor" (AF) performed on other plants [2] have not given clear-cut results. Attempts to ascribe this property to abscisic acid [3] have not yet undergone development. Only the role of ethylene and of compounds producing it in the process of lead-fall is not a matter of doubt [2, 4].

Nevertheless, some authors tend to consider that the AF is an integrated influence of the change in the levels of a whole set of compounds [2] plus the stress action of the environment, including day length [3]. We set ourselves the task of performing experiments permitting the combination of both directions of the searches – attempts to reveal a concrete carrier of the AF and a correlation of the levels of individual compounds of nonhormonal nature accessible for analysis in standard and selection lines.

---

Institute of the Chemistry of Plant Substances, Uzbek SSR Academy of Sciences, Tashkent. Institute of Experimental Plant Biology, Uzbek SSR Academy of Sciences, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, Vol. 6, pp. 796-805, November-December, 1989. Original article submitted January 17, 1989.

 0009-3130/89/2506-0676$12.50 

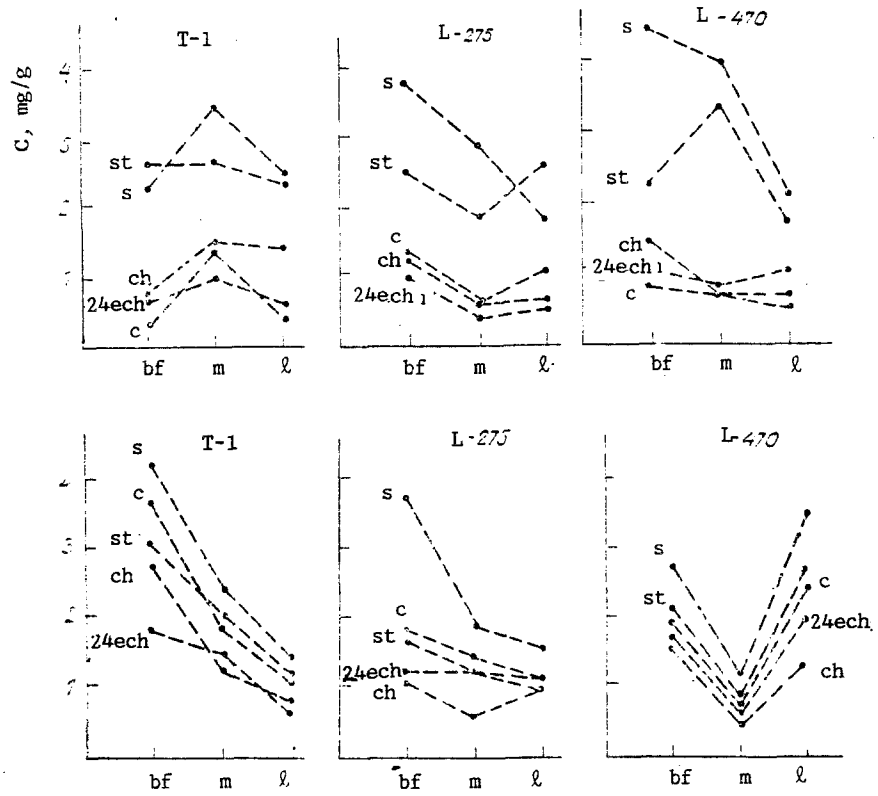


Fig. 1. Change in the amounts of free sterols (mg/g of air-dry weight) in the leaf blades (upper series) and petioles (lower series) of variety T-1 and lines L-275 and L-470 over the phases of development: bf – budding-flowering; m – maturation; ℓ – leaf-fall (s – sitosterol; st – stigmasterol; c – campesterol; ch – cholesterol; 24ech – 24-ethylidenecholesterol).

In the first stage of the search for the AF, the total extractive substances from the leaves of the cotton plant together with the petioles, collected in the period before natural leaf-fall were extracted, with the subsequent testing of individual fractions for defoliating activity and the analysis of the compositions of the active fractions. This experiment required particular care and the necessity of achieving reproducible results, the first of which are being accumulated and processed at the present time.

The second direction of the investigations consists in the quantitative and semiquantitative comparison of the amounts of substances in different selection lines of the cotton plant and a consideration of the results obtained using, as an example, the analysis of the free and bound sterols and triterpenoids.

The following information is available on the physiological functions of these groups of substances in plants. Heftmann [5] considers that free sterols – most frequently cholesterol – are precursors in the biosynthesis of the main types of physiologically active steroid molecules. On the other hand, it has been shown [6] that sterols are localized in the intracellular organelles, and their interaction with phospholipids stabilizes the membranes and controls their permeability. At the same time, it has been reported that branching of the side chain at C-17 decreases, and the appearance of π-bonds increases, the efficacy of the steroid molecule as a membrane stabilizer.

Esters of sterols and fatty acids (SEs) are also localized in the intracellular organelles [7] but they do not act as membrane stabilizers [8]. Their role amounts to the transport of the sterols [7]; however, it was shown in a later study that these compounds regulate the level both of free sterols and of unsubstituted fatty acids in different periods of ontogenesis, and they store and, at the necessary moment, liberate these compounds and, in addition to this, bind inactive sterols [9].

There is no definite information in the literature relative to the physiological functions of particular triterpenoids. It is known that squalene, and cycloartenol and some other compounds are biogenetic precursors of sterols [11]. In view of the nature of the activity of plant triterpenoids in relation to other biological materials (herbicidal, antimicrobial, antiinflammatory) it may be assumed that the role of their numerous varieties consists in the protection of the plant carrier from various ecological factors [12].

The unseparated sum of the extractive substances from leaf blades the age of which was an indication of the approach of the leaf-shedding process and from their petioles, including the so-called separating layer in which the reactions leading directly to mechanial shedding take place [3] were investigated. The biological materials obtained from the lower sections of plants of the control variety Tashkent-1 (T-1) and the selection lines L-275 and L-470 grown under identical conditions in one plot in three periods: budding-flowering, maturing, and leaf-fall.

Line L-275. Bush of bell-like form, medium-leafy, height of the main stem 120-130 cm, leaves large with 3-5 lobes, their fall taking place with the ripening of the adjacent bolls and ranging according to the year of growth from 60 to 75% on September 15.

Line L-470. Bush of bell-like form, medium-leafy, height of the main stem 115-120 cm, leaves of average size with 3-5 lobes, leaf fall taking place with the ripening of the bolls and ranging from 85 to 90%.

Variety T-1. Bush of pyramidal form, highly leafy, height of the main stem 115-130 cm, leaves of medium size with 3-5 lobes, leaf fall on September 15 ranging from 15-40%.

To analyze the sum of extractive substances we used various mass-spectrometric methods. General mass spectra of the whole sums showed no quantitative differences in the composition of the sterols and triterpenoids. The quantitative determination of the components was carried out by the method of multipeak monitoring [13]. Free sterols – sitosterol, campesterol, cholesterol, and stigamasterol – were determined, with the use of the corresponding standard solutions, from the peaks of the molecular ions with m/z 414, 400, 386, and 412. As the standard for calculating the amount of 24-ethylidenecholesterol we used an analogue of it – 24-methylenecholesterol. In this case, the analytical peak was the peak of the maximum ion with m/z 314, which is common for the two compounds [14]. The calculated amounts of the individual sterols ranged between 0.5 and 5 mg/g of air-dry material, i.e., from 0.05 to 0.5%.

The dynamics of the change in the amount of free sterols during the vegetation periods in the leaf blades and petioles of variety T-1 and the deciduous lines L-275 and L-470 are shown graphically in Fig. 1. The upper row of graphs characterizes the amounts of sterols in the leaf blades. Their change is represented by substantially different patterns in all three cases. A common feature is the considerable predominance of sitosterol and stigmasterol over the other sterols, which is characteristic of many plant materials. However, the most important feature must be considered to be the fact that the maxima of the accumulation of all the sterols (apart from stigamsterol) do not coincide with the phases for the control variety and the deciduous lines: in T-1 they appear in the maturation phase, and for L-275 and L-470 in the budding-flowering phase. The marked differences in the dynamics of the accumulation of stigmasterol in the two deciduous lines possibly indicate that the biosynthesis of this compound is subjected to the influence of the genome to the greatest degree.

Information exists that the ratio of the amounts of stigmasterol and sitosterol depends on the development of plants [10] and, in particular, the maturity of the leaves. In this paper it is reported that this ratio is higher in the older, lower, leaves and lower in the younger, upper, leaves, of the tobacco plant. Other authors have reported that stigmasterol plays the most important role in the later stages of the development of the cells [15].

Let us consider the graphs of hte upper row of Fig. 1 from the point of view of the ratio shown. In the case of L-275 the maximum ratio of stigmasterol to sitosterol is found in the leaf-fall phase, while in the leaf blades of L-470 it increases sharply even in the maturation phase and then remains approximately constant up the leaf-fall phase. In the standard variety T-1 in the same period the opposite tendency is observed: a decrease in the stigmasterol:sitosterol ratio up to the leaf-fall phase. Conversely, in the budding-flowering phase the amount of stigmasterol is higher than that of sitosterol.

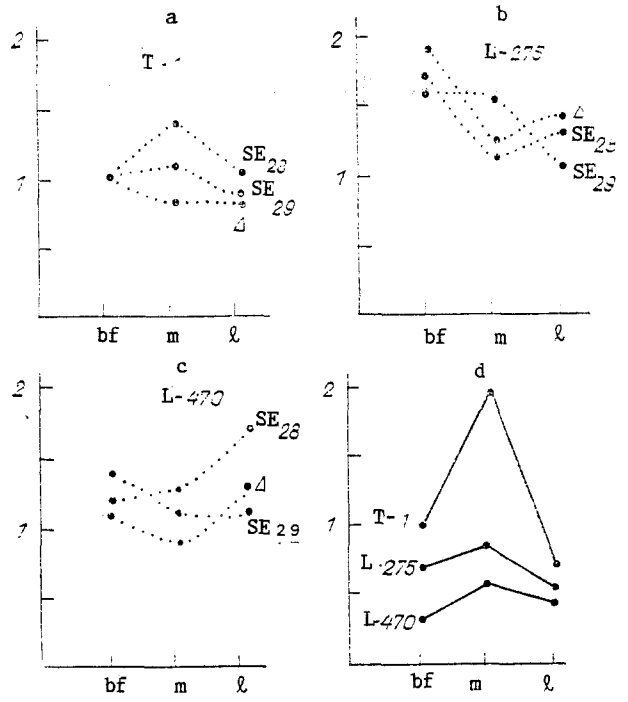


Fig. 2. Change in the relative amounts of esters of campesterol ($SE_{28}$) and sitosterol ($SE_{29}$) and of C-17 sterols unsaturated in the side chain (Δ) in the leaf blades of the three lines during the three phases of development (the amount of esters in the bf phase of T-1 was taken as unity) (a, b, c). The level of sitosterol esters in the petioles of the three lines over the three phases relative to the level in T-1 in the bf phase (d).

For another sterol unsaturated in the C-17 side chain – 24-ethylidenecholesterol – a minimum level is found in the ripening phase for L-275 and L-470, while in the control variety the maximum level of the sterol appears in this period.

A pattern completely different in nature is shown by the dynamics of the change in the level of free sterols in the petioles of the three samples (Fig. 1, lower row of graphs). For T-1 the level of all sterols, without exception, falls sharply up to the leaf-fall phase, for L-275 this process slows down between the two last phases, and in the same period the amount of these substances in the petioles of L-40 rises greatly. Thus, the dynamics of the change in the amounts of free sterols correlates with the level of maturity of the leaf that is characteristic for each line in the given period of collection of the material. It is most likely that the extractive substances of the petioles of T-1 and L-275 isolated from material collected later will also show a rise in the level of free sterols. We shall check this hypothesis later.

It can be seen from Fig. 1 that the amounts of free sterols in the two organs investigated were of the same order, while in individual cases – for example in the petioles of T-1 in the budding-flowering phase and in the petioles of L-470 in the leaf-fall phase – the concentration of free sterols was higher than in the leaf blades. However, one must take into account the difference in the weight of the air-dried leaf blades and petioles: in the case of T-1 one petiole weighed an average of 7 times, and in the case of L-470, 4.5 times less than one leaf blade. Consequently, the absolute level of any free sterol in a petiole amounted, according to our calculations, to 15-40% of its weight in a leaf blade. Thus, if it is considered that the biosynthesis of sterols takes place mainly in the leaf blade, their residue after migration to the petiole is sufficient for further biosynthetic transformations.

Let us consider, in particular, possible interconversions of the free sterols following from the upper row of graphs in Fig. 1.

The sharp decrease in the level of sitosterol during the whole period of observation in the leaves of L-275 and L-470 was accompanied by some increase in the amount of 24-ethylidenecholesterol, which shows the possibility of the conversion of the major sterol into minor ones. This contradicts schemes of biosynthesis according to which isomers of the latter

(fucosterol and isofucosterol) are, conversely, converted into sitosterol [16]. Judging from the same graphs, stigmasterol can also be biosynthesized from sitosterol, but this process takes place in different phases in different deciduous lines. According to the literature, no enzyme catalyzing this transformation has been found [17]; however, for the case of digitalis [18] it has been shown that this conversion can take place under natural environmental conditions.

In order, in some degree, to differentiate the biological functions of sterols saturated and unsaturated in the C-17 side-chain, we summed the amounts of both types in the leaf blades and petioles of T-1, L-275, and L-470 over the three vegetation periods. The results are shown graphically in Fig 2, a, d. It can be seen from graph (a) that the discussion on the shift on the maximum in the accumulation of free sterols in the leaf blades of the selection lines and the control relates wholly to the saturated sterols. So far as concerns the unsaturated sterols from the same materials (graph b), here a closeness of their levels in the initial phase and marked differences between the line in the dynamics of accumulation are observed. The main component of these totals is stigmasterol, and therefore the same contradiction is observed here as in the graphs of the first row of Fig. 1 — the natures of the change in the levels of sterols are similar for T-1 and L-470 but different for the deciduous line L-275.

However, this inexplicable contradiction relates only to the amount of unsaturated sterols in the leaf blade. So far as concerns the sum of saturated and unsaturated sterols of the petioles (graphs c and d), here there is a monotypical pattern with slight quantitative variations: a sharp fall in the amount of sterols in T-1, a retardation of this phenomenon in L-275 and rise up to the leaf-fall phase in the petioles of the L-470 line.

The absence of model samples of sterol esters with fatty acids forced us to estimate their amount by a relative method. We compared the total heights of the characteristic peaks of the selection lines obtained by the method of multipeak monitoring with the same values of the control variety T-1 taken as unity in the budding-flowering phase. The disadvantages of this method were partially compensated by the fact that the calculations of the absolute and relative amounts of all the substances being analyzed for each line and phase were made from a single experiment.

As characteristics we used the peaks of the $(M - RCOOH)^+$ ions with m/z 396 (esters of monounsaturated $C_{29}$ sterols), 394 (esters of diunsaturated $C_{29}$ sterols), and 382 (esters of monounsaturated $C_{28}$ sterols). Figure 2a-c shows the sums of the heights of these three peaks in the spectra of extracts of leaf blades of T-1, L-275, and L-470 in the three phases with respect to the sum of the heights of these peaks in the spectra of T-1 in the budding-flowering stage. In view of the selected form of illustrating the results and the absence of information on the relative sensitivity of the mass spectrometer to the three analytical peaks, it is impossible from the graphs of Fig. 2a-c, to judge the relative amounts of esters of various sterols, although the amount of sitosterol esters was undoubtedly higher than that of the other esters, since the height of the peak of the ion with m/z 396 far exceeded the heights of the peaks of the other ions. On the other hand, it is known that one and the same sterol can be esterified by a whole set of fatty acids [9], and therefore the graphs of Fig. 2a-c, permit only an approximate estimate of the relative distribution of the esters of one sterol over the three phases and the three samples of leaf material. It follows from the graphs, in the first place, that the initial level of SEs was higher in the deciduous lines than in T-1. The subsequent change in the level of esters of the various sterols took place differently. In T-1, sitosterol ($C_{29}$) esters accumulated in the ripening stage. In L-275, in the first stage their amount remained at the same level and then diminished partly up to the leaf-fall stage. In L-470 their level greatly decreased, so that the final amounts were similar in all the samples.

The maximum amount of campesterol ($C_{28}$) esters was found at different periods in the three cases. So far as concerns the SEs with $\pi$-bonds in the C-17 side-chain, for T-1, L-275, and L-470 their amount was lowest in the maturation stage.

In all cases, with rare exceptions, the amount of free sterols and their esters in the leaf blades changed in parallel. Thus, the idea of the storage and liberation of sterols through SEs was not confirmed here, and this all the more since, according to Grunwald's results [10], the levels of all bound forms of sterols in the leaves amount to not much more than 1/3 of the sum of the sterols. On the basis of the postulate of these sterols as biosynthetic precursors of SE [19] it may be assumed that a certain of the free sterols is constantly passing into the bound form.

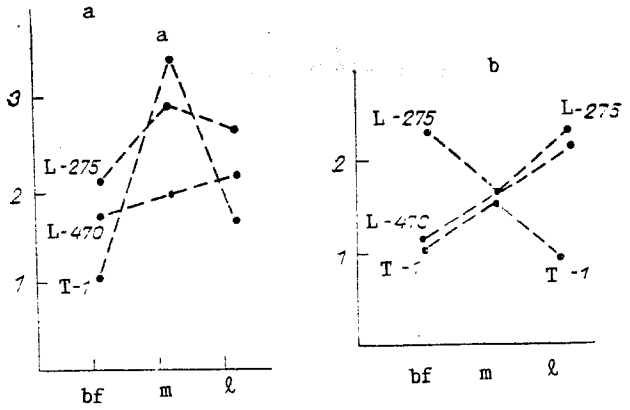


Fig. 3. Changes in the levels of amyrin (a) and its esters (b) in the leaves of the three lines over the three phases relative to the level of these components in T-1 in the bf phase.

We observed a completely different pattern in a comparison of the dynamics of the accumulation of free sterols in the petioles of the three lines (Fig. 1) with the relative change in the levels of sitosterol esters in the same materials (Fig. 2d). The initial levels of free and bound sitosterol in the budding-flowering phase was highest for T-1 and lowest for L-470. Then, on passing to the maturation phase, the level of free sitosterol fell sharply and the level of the bound sterol rose considerably (particularly in T-1), which may indicate a conversion of the former into the latter. Conversely, up to the leaf-fall phase the level of bound sitosterol fell and became approximately the same in all three samples.

The fact that the linked process of the liberation of free and the decrease in the amount of bound sterols took place most clearly in the petioles of the deciduous line L-470 possibly indicates a breakdown of the membranes. The subsequent fall in the level of free sterols in the petioles of T-1 can be explained by a continuing process of their binding with phospholipids. In the petioles of L-275 there was a superposition of the two processes and, therefore, the fall in the level of sitosterol slowed down. Thus, analysis of the graphs of Figs. 1 and 2 permits the conclusion that the sterols of the petioles, regardless of their structure, participate predominantly in membrane-building and membrane-breakdown processes. In addition to this process, the sterols of the leaf blades exhibit individual features, participating in biogenetic transformations more diverse in nature.

In the spectra of all the samples, a single molecular peak with m/z 426 corresponding to a free pentacyclic triterpenoid with the composition $C_{30}H_{50}O$ was observed which could belong to one of the isomers – amyrin, cycloartenol, or lanosterol – found most frequently in plant compounds of this class and composition. From the characteristic ion fragments in the spectra the peaks of ions with m/z 218 and 203 stood out by their high intensity, which permits the triterpenoid to be referred to the oleanene amyrin [20].

It can be seen from Fig. 3a, that the level of this substance was higher in the deciduous lines (the level in T-1 was taken as unity) in the budding-flowering phase, but by the maturation stage its level in the leaf blades of T-1 had risen sharply and had then fallen just as sharply. In the deciduous lines, the amount of amyrin had a weak tendency to rise in the leaf-fall stage as compared with the initial phase.

The bound amyrin was analyzed from the relative total height of the peaks of the ions $(M - ROH)^+$ with m/z 408. The change in the level of free amyrin and its esters (Fig. 3b) in T-1 and L-470 took place in parallel, and for L-275 in antiparallel. We report only the difference between the lines and do not comment on the reasons for these differences, since the physiological role of amyrin and its esters is unknown in this case.

## EXPERIMENTAL

The freeze-dried material (1 g) from the plants was extracted on a shaking machine first with 90 ml of alcohol and then with 90 ml of benzene. The extract was evaporated to dryness in a rotary evaporator at 40°C. The residue so obtained, after it had been brought to constant weight, was treated with redistilled chloroform in an amount of 1 ml per 1 mg of residue. The resulting solutions were used for mass-spectrometric analysis.

Below we give the weights in milligrams of the residues obtained from 1-g samples.

| Phase | | Leaf blades | Petioles |
|---|---|---|---|
| Budding flowering | T-1 | 80 | 140 |
| | L -275 | 170 | 110 |
| | L -470 | 120 | 110 |
| Maturation | T-1 | 150 | 90 |
| | L -275 | 150 | 90 |
| | L -470 | 130 | 90 |
| Leaf-fall | T-1 | 130 | 80 |
| | L -275 | 150 | 90 |
| | L -470 | 130 | 100 |

Preparation of Standard Solutions of the Sterols. Solution I: 0.140 mg of sitosterol in 7.0 ml of chloroform. Solution II: a mixture of 0.125 mg of sitosterol, 0.105 mg of stigmasterol, 0.110 mg of campesterol, 0.100 mg of sitosterol, and 0.150 mg of 24-methylenecholesterol in 5.9 ml of chloroform.

In turn, 2 μl of each of the total materials and 1 μl of standard II, after the solvents had been evaporated off, were introduced through a SVP5 direct-introduction system into a MKh 1310 mass spectrometer. The experiments with the total sample from the plant material were repeated not less than twice. A section of the mass spectrum in the interval of 300-450 a.m.u. was scanned 10-11 times with a smooth change in the temperature of the evaporator bulb from 80 to 200°C and a constant temperature of the ionization chamber of 170°C. The ionizing voltage was 50 V, the collector current 40 μA, the rate of scanning 25 a.m.u. per second (linear sweep), the speed of the recording chart 10 mm/s, R = 1200. In each scan the heights of all the characteristic peaks were measured and the corresponding heights were summed.

The sums of the heights of the characteristic peaks in the spectra of the standard II were used to determine the relative sensitivity of the mass spectrometer to each of the five free sterols. The relative sensitivities averaged over all the introductions of standard II had the following values: cholesterol – 1.0; campesterol – 0.73; stigmasterol – 0.58; sitosterol – 0.70; 24-methylenecholesterol – 0.63. By comparing the total height ($\Sigma h$) of the peaks ($\Sigma h_{st}$) and in the sample ($\Sigma h_s$) we determined the absolute amount of the sterol in the sample:

$$g_s = \frac{g_{st} \Sigma h_s}{\Sigma h_{st}}, \quad (1)$$

where $g_{st}$ and $g_s$ are the weights of the sterol in the standard and in the sample.

In addition to the calculation of the amount of each sterol by this method, the results obtained were checked in the following way, using relative sensitivities:

$$g_{sx} = g_s \frac{\sigma_s \cdot \Sigma h_x}{\sigma_x \cdot \Sigma h_s}, \quad (2)$$

where $g_{sx}$ is the amount of component x; $g_s$ is the amount of any component obtained from Eq. (1); $\sigma_s$ and $\sigma_x$ are the relative sensitivities; and $\Sigma h_s$ and $\Sigma h_x$ are the total heights of the corresponding peaks.

For an additional check we used the addition of 1 μl of standard I to 2 μl of the sample being analyzed, and the amount of sitosterol in the 2 μl was calculated from the formula

$$g_s = \frac{g_{st}(\Sigma h_{(st+s)} - \Sigma h_{st})}{\Sigma h_s}, \quad (3)$$

where $g_s$ is the amount of sitosterol in the 2 μl of sample; $g_{st}$ is the amount of sitosterol in the 1 μl of standard; $\Sigma h_{(st+s)}$ is the sum of the heights of the peaks of the mixture of the standard and sample; and $\Sigma h_{st}$ is the sum of the heights of the peaks of the 1 μl of standard.

All the results were averaged. The relative error of the determination was 10%.

The amount of free sterols in the air-dry material was calculated from the formula

$$c\left(\frac{\text{mg}}{\text{g}}\right) = \frac{S_S \cdot V}{v \cdot G}, \quad (4)$$

where v is the volume of sample being analyzed; V is the volume of the solution of extractive substances; and G is the weight of the air-dry material subjected to extraction.

To determine the relative amount of bound sterols and triterpenoids we used the total heights of the characteristic peaks corrected in the light of the yield of extractive substances from unit weight of air-dry material.

The samples of sterols were provided by L. S. Keifer (All-Union Scientific-Research Institute of Biological Methods of Plant Protection, Kishinev).

## SUMMARY

In a comparison of the amounts of free and bound sterols in the leaf blades and petioles of variety T-1 and the deciduous lines L-275 and L-470 in three phases of ontogenesis it has been established that in the leaf blades the maxima of the accumulation of free sterols saturated in the C-17 side-chain in the deciduous lines appears in an earlier phase than for T-1. The dynamics of the change in the level of unsaturated sterols are individual for each line, which is possibly a reflection of qualitative and quantitative differences in their biosynthetic transformations.

The nature of the change in the level of free sterols in the petioles is typical for each line and does not depend on the nature of the sterol, which may be associated with processes of the stabilization and breakdown of cell membranes.

As a rule, the levels of free and bound sterols in the leaf blades change in parallel, but in the petioles between the first and second phases they change in antiparallel.

## LITERATURE CITED

1. A. A. Zhuchenko, The Genetics of Tomatoes [in Russian], Shtiintsa, Kishinev (1973), p. 438.
2. P. F. Wareing and I. D. J. Philipps, Growth and Differentiation in Plants, Pergamon, Oxford (1981) [Russian translation, Mir, Moscow (1984), p. 442.
3. A. W. Galstone, P. J. Davis, and R. L. Satter, The Life of the Green Plant, Prentice-Hall, Englewood Cliffs, N.J. (1981) [Russian translation, Mir, Moscow (1983), p. 313.
4. K. Doerffing, Plant Hormones [Russian translation], Mir, Moscow (1985), p. 217.
5. E. Heftmann, in: Isopentenoids in Plants (ed. W. D. Nes et al.,), Marcel Dekker, New York (1984), p. 487.
6. E. Heftmann, Phytochemistry, 13, 891 (1975).
7. R. J. Kemp, L. J. Goad, and E. I. Mercer, Phytochemistry, 6, 1609 (1967).
8. C. Grunwald, Plant Physiol., 48, 653 (1971).
9. B. D. Whitaker and D. L. Nelson, Lipids, 22, 386 (1987).
10. C. Grunwald, Phytochemistry, 14, 79 (1975).
11. P. Benveniste, L. Hirth, and G. Ourisson, Phytochemistry, 5, 31 (1966).
12. V. A. Paseshnichenko, Advances in Science and Technology. Biological Chemical Series [in Russian], VINITI, Moscow, Vol. 25 (1987), p. 6.
13. E. G. Sirotenko, Ya. V. Rashkes, and S. K. Usmanova, Khim. Prir. Soedin., 532 (1989).
14. C. Djerassi, Pure Appl. Chem., 50, 171 (1978).
15. E. Duperon, C. R. Acad. Sci. Paris Ser. D., 266, 1658 (1971).
16. E. Heftmann, Steroid Biochemistry, Academic Press, New York (1970) [Russian translation], Mir, Moscow (1972), p. 26.
17. J. A. Waters and D. F. Johnson, Arch. Biochem. Biophys, 112, 387 (1965).
18. R. D. Bennett and E. Heftmann, Steroids, 14, 403 (1969).
19. G. Adler and L. Kasprzyk, Phytochemistry, 14, 627 (1975).
20. H. Budzikiewicz, J. M. Wilson, and C. Djerassi, J. Am. Chem. Soc., 85, 3688 (1963).