ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 56, nr 2: 303-314
1987

# The effect of the plant growth retardants AMO-1618 and CCC on the synthesis of ribonucleic acids and proteins in triticale embryos during the initial phase of germination

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(Received: August 14, 1986. Accepted: October 9, 1986)

## Abstract

Triticale var. Grado caryopses were subjected to imbibition and germination in the presence of the growth retardants, AMO-1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidinecarboxylate methyl chloride) and CCC (2-chloroethyl)-trimethylammonium chloride) at the following concentrations, $3 \times 10^{-4}$ M and $10^{-3}$ M. These compounds exerted a very strong inhibitory effect on the initiation of germination processes, growth of embryos and the germination capacity of the caryopses. At the concentration of $10^{-3}$ M, AMO-1618 showed an especially strong effect, lowering the germination capacity of the caryopses to about 50%. It was also shown that both retardants are decidedly more effective on the germination of whole, intact caryopses than on that of isolated embryos. During the very earliest hours of germination, these retardants already inhibited RNA synthesis. The participation of the polyribosome fraction in the total ribosome fraction of embryos in the control sample after 24 hrs of germination of caryopses equalled about 70%, while in the samples treated with CCC ($10^{-3}$ M) — about 57%, in the samples treated with AMO-1618 ($10^{-3}$ M) — about 35%. The inhibition of incorporation of $^{14}$C-amino acids into ribosomal proteins in the polyribosome fraction was in the case of CCC about 13%, while in the samples treated with AMO-1618, about 55%. In the monosome fraction (80S), the inhibition by CCC was about 23%, whereas in the samples treated with AMO-1618 it reached around 73%. From this data it is evident that the studied retardants have a significant influence on the synthesis of ribonucleic acids as well as on ribosome proteins. These results also suggest the existance of another mechanism, aside from that of inhibition of gibberellin biosynthesis, inhibiting the growth and development of cells. The high percentage of ribosome subunits in the samples treated with CCC,

in comparison with controls and samples treated with AMO-1618, points to different mechanisms by which these two compounds affect protein biosynthesis.

*Key words: AMO-1618, CCC, ribonucleic acid synthesis, ribosome protein biosynthesis*

## INTRODUCTION

Many synthetic growth retardants, sold under various commercial names, have been discovered during recent years. Some of them are of great importance to agriculture and horticulture. Their use brings about, among others, the inhibition of elongation of the shoots of decorative plants, due to which the plants attain more desired traits. Growth retardants are also used to shorten and strengthen the stems of certain cultivated species, especially wheat, which limits its flattening in the field. There are also many other applications for growth retardants, e.g. the intensification of flowering, formation of fruit, rooting of cuttings, formation of tubers and bulbs and increasing the resistance of plants to drought, cold and soil salinity. The physiological and biochemical mechanisms by which all of these effects are obtained, remain unknown. The compounds studied in this report, AMO-1618 and CCC, inhibit gibberellin synthesis in the plants on which they have been used (Dennis et al. 1965, Zeevaart 1966). Even in the case of these two retardants, to which probably the most attention has been paid, much uncertainty and contradictory information on their effect on cell metabolism still remain (Gräser 1977, Bode and Wild 1984). This may be due to the different ways in which the retardants were introduced into the plant, the different species and varieties used in the experiments and the various conditions under which the plants grew and developed.

The aim of this study was to examine the effect of AMO-1618 and CCC on the synthesis of ribonucleic acids and ribosome proteins and on the process of formation of polyribosomes in embryos in the first stages of germination of triticale caryopses. In this study, the effect of these compounds on the germination of isolated embryos and whole, intact caryopses was also examined. Further studies on the mechanism of AMO-1618 and CCC action will be presented in a later publication.

## MATERIAL AND METHODS

The experiments were conducted on triticale var. Grado caryopses supplied by the Plant Cultivation Station in Choryń. The caryopses were washed with tap-water and placed in a 1% solution of sodium hypochloride

for 3 minutes. After sterilization, the caryopses were rinsed with sterile water and surface dried. The sterilized material was then allowed to imbibe for 12 hours at a lowered temperature. The procedure was as follows: 300 caryopses were placed in Petri dishes laying on ice, and cooled sterile water was poured on them so that they were half-covered. The dishes were then transferred to a cold-room with a temperature of +2°C. In the appropriate samples, imbibition was in the presence of $3 \times 10^{-4}$ or $10^{-3}$ M solutions of the retardants AMO-1618 (2-isopropyl-4-dimethylamino-5-methyl-phenyl-1-piperidinecarboxylate methyl chloride, Serva) and CCC (2-chloroethyl) trimethylammonium chloride, BDH).

After preimbibition, the swolen caryopses were germinated in Petri dishes in the dark at a temperature of 21-22°C. Germination was in the presence of chloramphenicol (10 μg $cm^{-1}$), the appropriate retardant and 5-$^{3}$H-uridine or $^{14}$C-protein hydrolysate (0.8 MBq $cm^{-3}$). The radioactive precoursors were obtained from Chemapol UVVVR, Czechoslovakia. After 3 or 24 hours of germination, embryos were isolated from caryopses cooled on ice, the remaining, non-metabolised precursors were rinsed from the surface of the material, which was then dried and stored at a temperature of −25°C in closed vials for further analysis.

The total ribosome fraction (polyribosomes + monosomes + ribosome subunits) was isolated from the embryos by the methods described previously (Weidner 1984b). About 2 g of the plant material were homogenized in buffer "A" (0.2 M sucrose, 200 mM Tris-HCl (pH 8.5), 30 mM $MgCl_2$, 60 mM KCl). The homogenate was centrifuged at 29000 × g. Next, the supernatant was transferred to the 65 Ti rotor test tube on the 4 $cm^3$ layer of 1.5 M sucrose in buffer "B" (40 mM Tris-HCl (pH 8.5), 10 mM $MgCl_2$, 20 mM KCl) and centrifuged in a Spinco L-3-40 ultracentrifuge at 95000 × g for 90 minutes. The pellet (about 1 mg) was suspended in 1 $cm^3$ of buffer "B" and overlaid on the sucrose concentration gradient. Polyribosomes were fractionated by centrifugation at 122000 × g in SW-41 rotor for 75 minutes. In order to assay the amount of ribosomes, it was assumed that the extinction of a 1% solution of ribosomes in a cuvette with a 1 cm light path, at 260 nm equals $E_{1\%}^{1cm} = 135$ (Gualerzi and Cammarano 1969).

Isolation and fraction of total RNA was done according to Tanifuji et al. (1970), as modified by Takaiwa and Tanifuji (1978). A sample of 200 embryos was homogenized in 10 $cm^3$ of buffer "A" of the following composition: 0.1 M Tris-HCl (pH 9.0) containing 0.1 M NaCl, 1% bentonite, 2% SDS and 100 μg $cm^{-3}$ polyvinyl sulphate. An equal volume of a mixture of m-cresol-phenol-water (10:70:20, v/v/v) and 8-hydroxyquinoline at a final concentration of 0.1% was added to the homogenate. The suspension was shaken and after centrifugation (5000 × g), the water phase was collected. This phase was then deproteinized three times with a mixture of chloroform-

-phenol, and ribonucleic acids precipitated with cold ethanol. After being dissolved, the RNA preparation was ultracentrifuged in a linear sucrose concentration gradient (5-20%) containing 25 mM Tris-HCl (pH 9.0), 50 mM NaCl and 5 mM EDTA (ethylene diamine tetraacetic acid). The samples were centrifuged for 5 h at 196000 × g in a Spinco L-3-40 ultracentrifuge in a SW-41 rotor. After centrifugation, the contents of a tube were partitioned into about 40 fraction and their extinction was measured at 260 nm. The RNA was then precipitated with an equal volume of 10% trichloroacetic acid and collected on glass fibre filters.

Radioactivity measurements for both of the methods used were carried out in a Beckman LS-1801 liquid scyntillation counter. All of the results presented in this study are the mean values of 3-5 separate experiments.

## RESULTS AND DISCUSSION

Triticale caryopses were subjected to imbibition and germination in water (control) and in solutions of retardants. The effect of the retardants was small until the 12th hour of imbibition, although it was found that the embryos from caryopses imbibing and germinating in the presence of AMO-1618 and CCC had a smaller weight (Fig. 1A). During further germination (12-24 h), the fresh weight of the embryos in the control sample grew very quickly and evident inhibition of fresh weight increase by both retardants was noted. At both concentrations used ($3 \times 10^{-4}$ and $10^{-3}$ M), AMO-1618 showed significatly greater inhibition than CCC (at the same concentrations).

Bode and Wild (1984) studied in detail the effect of CCC ($10^{-2}$ M) on the development of 11 day-old wheat seedlings. This retardant caused the immediate arrest of root growth and strongly inhibited the growth of leaves.

The effect of the retardants on the germination capacity of whole, intact caryopses is presented on Fig. 1B. These compounds caused significant delays in the initiation of germination and lowered the germination capacity of the grain to a large degree. In these studies, AMO-1618 at $10^{-3}$ M showed exceptionally strong inhibition, lowering the germination capacity of the caryopses to about 50%. The effects of the retardants on the germination of isolated embryos are presented on Fig. 1C. A significant influence of these compounds on the initiation and germination capacity of isolated embryos was found, similarly as in the experiments on whole caryopses. It should, however, be added, that the inhibition was much weaker than in the case of whole, intact caryopses.

It is generally accepted that in germinating cereal caryopses, GA is synthesized by the scutellum or embryo, and then diffuses to the aleurone

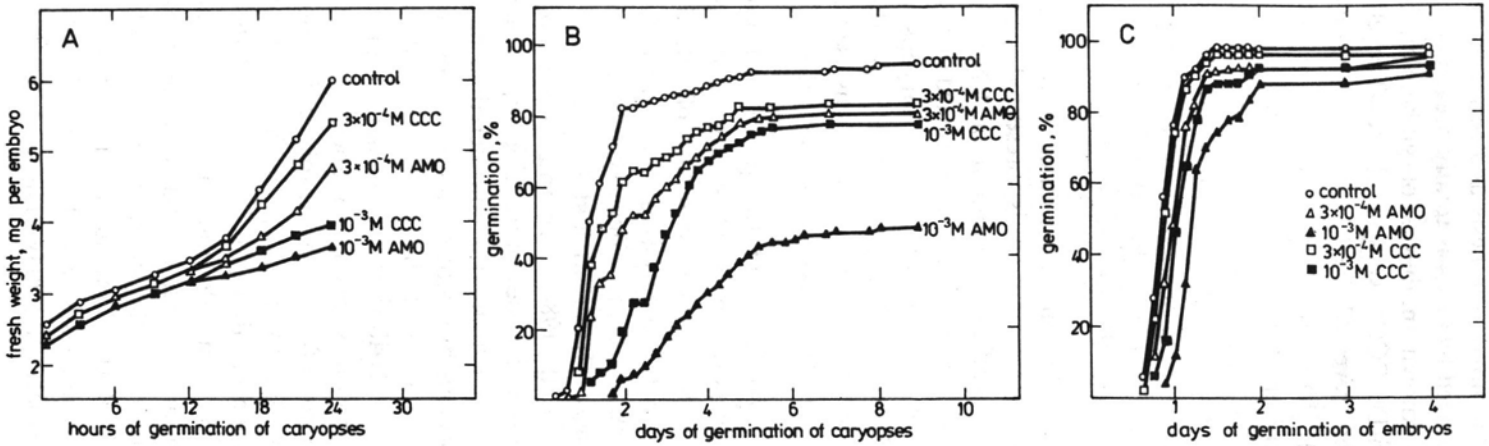


Fig. 1. The effect of the retardants AMO-1618 and CCC on: A — the increase of fresh weight of embryos in the initial phase of germination, B — germination capacity of whole caryopses, C — germination capacity of isolated embryos. The germination of grain and isolated embryos was preceded each time by 12 hours of imbibition of caryopses at a reduced temperature in the presence of the appropriate retardant. All of the data presented on the figures are the mean values of 5 independent experiments

layer, where it stimulates the "de novo" synthesis and secretion of α-amylase. This synthesis of α-amylase, induced by $GA_1$, is totally prevented by treatment of barley caryopses with all known inhibitors of gibberellin biosynthesis, such as: AMO-1618, Phosfon-D, CCC, Ancymidol etc. The effects caused by these retardants could be reversed each time by the use of $GA_4$ (Atzorn and Weiler 1983). This example explains to a certain degree the reason for the significantly greater inhibition by the studied retardants of the germination of whole caryopses than of isolated embryos. It must, however, be emphasized that the embryo isolated from the grain is also sensitive to the retardants (inhibiting gibberellin synthesis) (Fig. 1C) and to treatment with exogenous GA (Weidner 1984a). This points to the existence of still another mechanism of stimulation of germination by GA.

The effect of AMO-1618 and CCC on the synthesis of total and ribosomal RNA after 3 and 24 hours of germination is presented on Fig. 2 and Table 1. In these experiments, significant inhibition of RNA synthesis (on the basis of incorporation of $^3$H-uridine) by both retardants was already visible in the earliest hours of germination of the caryopses (Table 1). The inhibition was higher the higher were the concentrations of the retardants during preimbibition and germination. At both concentrations used, AMO-1618, caused significantly greater inhibition of RNA synthesis than CCC.

The effects of AMO-1618 and CCC on the process of polyribosome formation in embryos and incorporation of $^{14}$C-amino acids into embryo ribosome proteins during the 24 hour-long germination of triticale caryopses are presented on Fig. 3 and Table 2. Analysis of the sedimentation profile of polyribosomes gives information on the efficiency of translation and the entire process of protein synthesis (Romani and French 1977, Weidner and Wielgat 1983, Nowak et al. 1984, Fehling and Weidner 1986). The proportion of polyribosomes in the entire ribosome fraction (polyribosomes + monosomes + ribosome subunits) is considered to be a foolproof indicator of the intensity of protein biosynthesis under various experimental conditions (Nowak et al. 1984). Both of the retardants used in this study changed in a significant way the polyribosome sedimentation profile (Fig. 3). In the control sample, polyribosomes made up about 70% of the total ribosome fraction of the embryos after 24 hours of germination, whereas in the sample treated with CCC, about 57%. It should be emphasized that AMO-1618 at the same concentration ($10^{-3}$ M) lowered the percentage of polyribosomes to about 35%. In addition to the differences in the intensity with which both inhibitors acted, qualitative differences were also clearly visible in the sedimentation profiles of the ribosome fraction (Fig. 3, Table 2). The large proportion of ribosome subnits in the samples treated with CCC (approx. 13.4%) in comparison with the control and AMO-1618 samples (approx. 5 and 4.5%, respectively), may indicate different mechanisms

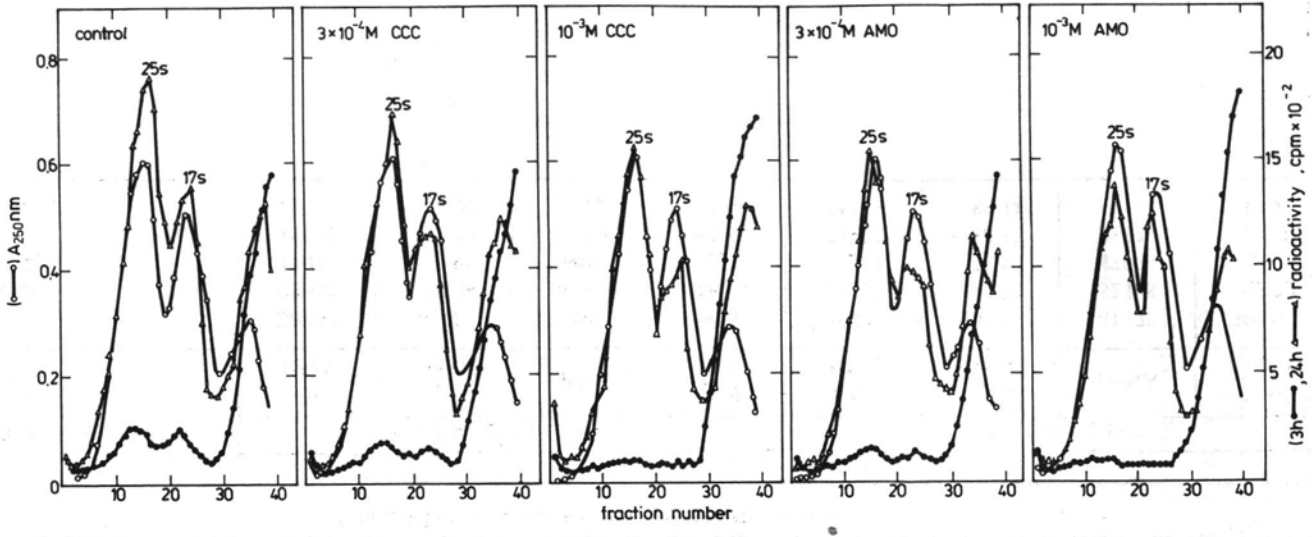


Fig. 2. The sedimentation profiles of total RNA (in a 5-20% sucrose gradient) isolated from triticale embryos. The caryopses were allowed to imbibe for 12 hours and to germinate for 3 or 24 hours. The preimbibition at a reduced temperature and germination were conducted in water (control) or the appropriate concentration of the retardants (AMO-1618 and CCC), after which the embryos were excised from the carypses and used for isolation of RNA. $^{3}$H-uridine (0.8 MBq cm$^{-3}$) and chloramphenicol (10 μg cm$^{-3}$) were also used during germination

Table 1

The effect of the retardants AMO-1618 and CCC on the synthesis of total and ribosomal RNA (25S+17S RNA) in embryos after 3 or 24 hours of germination of triticale caryopses

| Treatment | cpm mg $^{1}$ RNA | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3 h germination | | | | 24 h germination | | | |
| | total RNA | % of control | rRNA | % of control | total RNA | % of control | rRNA | % of control |
| Control | 20432 | 100.0 | 9008 | 100.0 | 85432 | 100.0 | 61178 | 100.0 |
| $3 \times 10^{-4}$M AMO | 18324 | 89.68 | 6188 | 68.69 | 72803 | 85.22 | 52188 | 85.30 |
| $10^{-3}$M AMO | 14911 | 72.98 | 4844 | 53.77 | 66231 | 77.52 | 47928 | 78.34 |
| $3 \times 10^{-4}$M CCC | 18842 | 92.22 | 6268 | 69.58 | 73612 | 86.17 | 54738 | 89.47 |
| $10^{-3}$M CCC | 15731 | 76.99 | 5017 | 55.69 | 71543 | 83.74 | 51137 | 83.59 |

by which both compounds act on the biosynthesis of protein. The synthesis of ribosomal proteins (on the basis of incorporation of $^{14}$C-amino acids) was also highly inhibited by the retardants. The different level of inhibition of protein biosynthesis in the individual fractions should be emphasized as should the exceptionally high activity of AMO-1618 on this process. The inhibition of incorporation of $^{14}$C-amino acids into ribosome proteins in the polyribosome fraction was in the case of CCC, about 13%, and in the sample treated with AMO-1618 — about 55%. While, in the monosome fraction (80 S), the inhibition caused by CCC was 23%, and in the samples treated with AMO-1618 reached 73% (Fig. 3, Table 2).

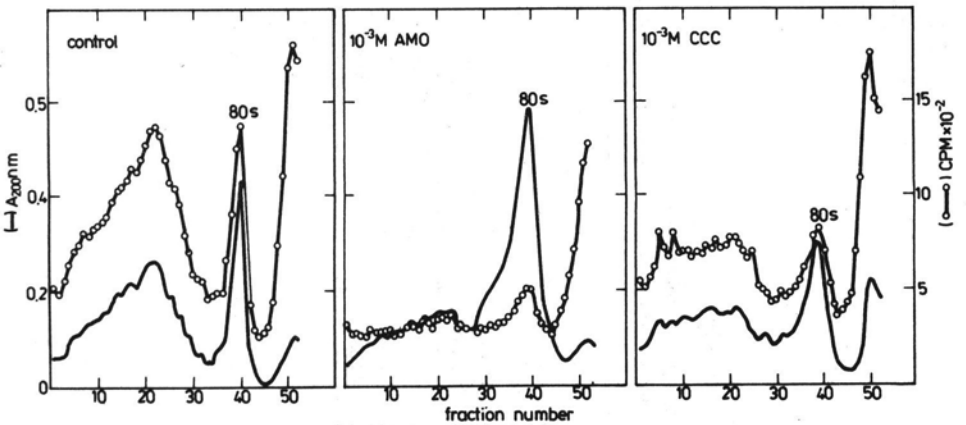


Fig. 3. The sedimentation profiles of polyribosomes (in a 12.5-50% sucrose gradient) isolated from triticale embryos. The caryopses were subjected to preimbibition (12 h) and 24 h germination. Preimbibition and germination were conducted in water (control) and in the presence of the retardants AMO-1618 and CCC (at a concentration of $10^{-3}$ M), then embryos were excised from the caryopses and used for the isolation of the total ribosome fraction. During germination, $^{14}$C-amino acid hydrolysate (0.8 MBq cm$^{-3}$) and chloramphenicol (10μg cm$^{-3}$) were also used

It can be concluded from these results that the studied retardants exert a significant influence on both the synthesis of ribonucleic acids and the synthesis of ribosomal proteins. Bode and Wild (1984) found that retardants also inhibit the synthesis of DNA.

The results of these studies and data from literature make it possible to believe that the effect of the retardants — especially at the higher concentrations ($10^{-3}$-$10^{-2}$ M), does not limit itself only to inhibition of the synthesis of gibberellins and the associated with this inhibition of the elongation of cells. The retardants act on many cellular processes and the reactions may be different depending on the type of plant material and different conditions of growth and development of plants (Gräser 1977).

Table 2

The effect of the retardants AMO-1618 and CCC on the formation of polyribosomes in embryos and on the incorporation of $^{14}$C-amino acid hydrolysate into embryo ribosome proteins durnig 24 h long germination of triticale caryopses

| Treatment | Changes in the percentage of ribosome fractions | | | cpm 1 mg $^{1}$ ribsome fractions | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | polyribo-somes | monori-bosomes | ribosome subunits | total ri-bosome fraction | % of control | polyri-bosomes | % of control | monori-bosomes | % of control |
| Control | 69.90 | 25.12 | 4.98 | 58430 | 100.0 | 65112 | 100.0 | 49241 | 100.0 |
| 10 $^{3}$M AMO | 35.10 | 60.39 | 4.51 | 24846 | 42.52 | 29113 | 44.71 | 13500 | 27.42 |
| 10 $^{3}$M CCC | 56.72 | 29.84 | 13.44 | 48687 | 83.32 | 56700 | 87.08 | 37815 | 76.80 |

Returning to the analysis of the sedimentation profile of polyribosomes (Fig. 3), it seems that the second mechanism of action of both retardants (not connected with GA) may also be different for AMO-1618 and CCC. The high proportion of ribosome subunits in the samples treated with CCC indicates that this compound is already active on the level of association of subunits into 80S ribosomes. More data on this will be presented in the next paper.

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*Wpływ retardantów wzrostu AMO-1618 i CCC na syntezę kwasów rybonukleinowych i białek w zarodkach triticale w początkowym okresie kiełkowania*

Streszczenie

Ziarniaki triticale odmiany Grado poddawano imbibicji i kiełkowaniu w obecności retardantów wzrostu AMO-1618 (metylo-chlorku kwasu 2-izopropylo-4-dimetyloamino-5-metylofenylo-1-piperydyno karboksylowego) oraz CCC (chlorku 2-chloroetylotrimetyloaminowego) w stężeniach $3 \times 10^{-4}$ M oraz $1 \times 10^{-3}$ M. Związki te wywierały silnie inhibicyjny wpływ na inicjację procesów kiełkowania, wzrost zarodków oraz na zdolność kiełkowania ziarniaków. Szczególnie silne działanie wykazał AMO-1618 w stężeniu $10^{-3}$ M, który obniżał zdolność kiełkowania ziarniaków do ok. 50%. Wykazano również, że oba retardanty działają znacznie silniej na kiełkowanie całych nie naruszonych ziarniaków niż na kiełkowanie izolowanych zarodków. Stosowane retardanty już w najwcześniejszych godzinach kiełkowania ziarna, hamowały syntezę RNA. Udział polirybosomów w ogólnej frakcji rybosomalnej zarodków w próbie kontrolnej wynosił po 24 h kiełkowania ziarna ok. 70%, w próbach traktowanych CCC ($10^{-3}$ M) — ok. 57%, a w próbach traktowanych AMO-1618 ($10^{-3}$ M) — ok. 35%. Inhibicja wcielania $^{14}$C-aminokwasów do białek rybosomalnych we frakcji polirybosomów wynosiła w przypadku stosowania CCC ok. 13%, natomiast w próbach traktowanych AMO-1618 — ok. 55%. Dla frakcji monosomów (30S) inhibicja wywołana przez CCC wynosiła ok. 23%, a w próbach traktowanych AMO-1618 aż ok. 73%. Z przedstawionych danych wynika, że badane retardanty wywierają istotny wpływ zarówno na syntezę kwasów rybonukleinowych, jak i na biosyntezę białka rybosomalnego. Uzyskane wyniki sugerują również istnienie, oprócz inhibicji biosyntezy giberelin, innego mechanizmu hamującego wzrost i rozwój komórek. Duży udział podjednostek rybosomalnych w próbach traktowanych CCC, w porównaniu z kontrolą i próbami traktowanymi AMO-1618, wskazuje na inny sposób oddziaływania tych dwóch związków na biosyntezę białka.