ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. XXXVI — nr 3
1967

Acenaphthylmercaptoalkanocarboxylic acids as plant growth substances

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The action of acenaphthene on cell division processes is very similar to that of colchicine (Shmuck 1938). During cell division c-mitotic anomalies occur which induce polyploidy in plants (Gavaudan and Durand 1938; Nebel 1938; Kostoff 1938; Shmuck and Gusseva 1939; Fukushima 1939; Clarke and Edwards 1962; Edwards 1962). A c-mitotic division results in the suppression of root growth. The observations showed that not all plants responded to the action of acenaphthene to an identical degree. And thus, the influence of acenaphthene on the roots of *Pisum* proved less effective than on the roots of *Allium* (Shmuck and Gusseva 1939; Edwards 1962). Recently, Fischer (1962) found giant forms of pollen grains in beet stems whose tips had been treated with acenaphthene solution.

Edwards (1962) started investigations on the physiological activity of a series of alcohol compounds—acenaphthene derivatives. Among the compounds examined by him, 3-(3-acenaphthyl propanol, 3-(5-acenaphthyl) propanol and acenaphthene were found to cause an inhibition of growth of *Pisum* seedlings and mitotic anomalies.

It should be stressed that, so far, the effect of acenaphthylmercaptoalkanocarboxylic acids on plants has not been examined. In order to extend the observations of the physiological activity of those acids, experiments were carried out to estimate their influence on seed germination and growth of lettuce seedlings. These experiments were justified because of the possible application of acenaphthylmercaptoalkanocarboxylic acids as likely plant growth regulators.

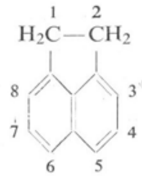
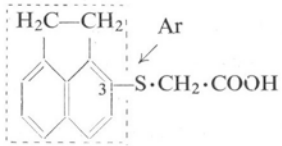
MATERIAL AND METHODS

The methods of obtaining acenaphthylmercaptoalkanocarboxylic acids (Table 1) have been worked out in the Department of Organic Chemistry, M. Curie-Skłodowska University, Lublin (Janczewski and Podkościelny 1958; 1959).

Free acenaphthylmercaptoalkanocarboxylic acids are readily soluble in nearly all organic solvents. Their sodium and potassium salts crystallize and are poorly soluble in water. Acids were applied in 0.01 mg/ml concentration in the following way. The 10-mg acid sample was dissolved in 1.3 ml of 0.1 N NaOH, neutralized with 1 ml of 0.1 N HCl, and made up to a 1 — l. volume with bidistilled water. Because of the low solubility of acenaphthene in water (less than 250 μg/l.; Edwards et al. 1961), it was used as a saturated solution. NaOH and HCl were added to control plants, grown in water and the acenaphthene solution, in a concentration identical with that of acid solutions.

Table 1

Acenaphthylmercaptoalkanocarboxylic acids

Name	Constitutional formula	Molecular weight	Melting point
Acenaphthene		154.20	95°C
3-Acenaphthylthioglycolic acid		244.30	143—4°C
3-Acenaphthylthio-(α-methyl)--glycolic acid	Ar—S·CH(CH₃)·COOH	258.33	117°C
3-Acenaphthylthio-(α-ethyl)--glycolic acid	Ar—S·CH(C₂H₅)·COOH	272.35	91°C
	Ar — acenaphthene ring		

The seeds of *Lactuca sativa* L., Böttner's variety, lot 306, from of the 1964 harvest were examined. Two types of tests were made.

1. In the experiments concerned with germination, 300 seeds were sown on Petri dishes 15 cm in diameter. The dishes were previously lined with two layers of Whatman 1 paper. The paper and the seeds were imbibed with 10 ml of the solution under test. Dishes were put in the thermostat. After the seeds had been saturated for 7 hours with suitable solutions, they were taken out of the thermostat and exposed to red light for 5 minutes. The control series was kept in darkness. The source of light was a 150 W bulb in a reflector lamp supplied with an interference filter, with light transmittance of 630 mμ wavelength. The light intensity, 43×10^2 erg/cm^{-2}sec^{-1}, was measured with an actinometer. The distance of the light from the level of the seeds was 45 cm. After exposure the dishes were placed again in the thermostat and the percentage of seeds which had germinated was calculated after 48 hrs.

2. In the experiments performed to estimate probable growth effects, 100 seeds were sown on Petri dishes to avoid excessive density. Except for the number of seeds, the treatment of seeds and the test conditions were identical. After four days of germination, 2 ml of bidistilled water was added to each Petri dish to maintain suitable humidity of the filter paper. The experiments ended when the seedlings had grown for 8 days. The plants were taken from the dishes at random. The length of roots and hypocotyls as well as the fresh weight of plants were determined.

In both types of experiments the seeds were imbibed, they germinated and grew in the same thermostat in darkness, at 25°C. All the necessary manipulations were performed under dim green light. In each series the experiments were repeated at least 3 times. The results were assessed statistically (Snedecor 1956).

RESULTS AND DISCUSSION

1. Effect of acenaphthylmercaptoalkanocarboxylic acids on the germination of lettuce seeds. The results of the above described experiments (Table 2) showed that the acids in question promote dark germination of lettuce seeds as compared with those treated with acenaphthene and with the water control. The promoting effect proved even more pronounced with lettuce seeds exposed to red light.

Table 2

Effect of acenaphthylmercaptoalkanocarboxylic acid on germination of lettuce, seeds

Solution	Number		Germination, %	
	Experiments	Replications	Darkness	Light
Water	4	12	61±1.398	74±1.623
Acenaphthene	3	9	57±1,726	68±1.060
3-Acenaphthylthioglycolic acid	3	9	85±0.807	91±0.870
3-Acenaphthylthio-(α-methyl)-glycolic acid	3	9	83±1.611	91±1.105
3-Acenaphthylthio-(α-ethyl)-glycolic acid	3	9	68±0.972	83±1.363

Since acenaphthene did not promote the germination of seeds, it is suggested that the side chain of the acid and not its acenaphthene ring is responsible for the germination promoting effect. The data referring to water germinated controls corroborate our earlier observations (Gawroński, in press) that red light of 630—660 mμ wavelength promotes germination of lettuce seeds (*Lactuca sativa* L., Böttner's variety, lot 306).

2. Effect of acenaphthylmercaptoalkanocarboxylic acids on the growth of lettuce seedlings. Preliminary experiments showed that the roots of lettuce seedlings treated

Table 3

Effect of acenaphthylmercaptoalkanocarboxylic acids on growth of lettuce seedlings

Solution	Plants examined		Dark		Light	
	Number	Part	Length, mm	Fresh weight, mg	Length, mm	Fresh weight, mg
Water	130	Whole	60 ±2.380	19.3 ±0.434	61 ±1.527	20.3 ±0.567
		Root	34 ±1.353	2.4 ±0.100	35 ±0.912	2.7 ±0.071
		Hypocotyl	26 ±0.912	16.9 ±0.534	26 ±0.577	17.6 ±0.645
Acenaphthene	130	Whole	69 ±0.408	20.9 ±0.200	69 ±0.577	21 ±0.300
		Root	36 ±0	2.5 ±0.057	35 ±0.707	2.7 ±0.071
		Hypocotyl	33 ±0.408	18.4 ±0.200	34 ±0.408	18.3 ±0.259
3-Acenaphthyl-thioglycolic acid	130	Whole	83 ±2.081	19.6 ±0.567	89 ±1.825	20.5 ±0.406
		Root	57 ±1.353	3.4 ±0.200	62 ±0.577	3.7 ±0.081
		Hypocotyl	26 ±0.408	16.2 ±0.768	27 ±1.000	16.8 ±0.533
3-Acenaphthyl-thio-(α-methyl)--glycolic acid	130	Whole	80 ±4.123	19.9 ±0.316	80 ±3.391	20.7 ±0.040
		Root	55 ±3.214	3.8 ±0.261	54 ±4.582	3.8 ±0.200
		Hypocotyl	25 ±0.707	16.1 ±0.040	26 ±0.912	16.9 ±0.234
3-Acenaphthyl-thio-(α-ethyl)--glycolic acid	130	Whole	76 ±5.773	20.7 ±0.267	79 ±5.507	21.0 ±0.400
		Root	50 ±4.847	4.4 ±0.234	53 ±4.847	4.5 ±0.141
		Hypocotyl	26 ±0.408	16.3 ±0.057	26 ±1.000	16.5 ±0.141

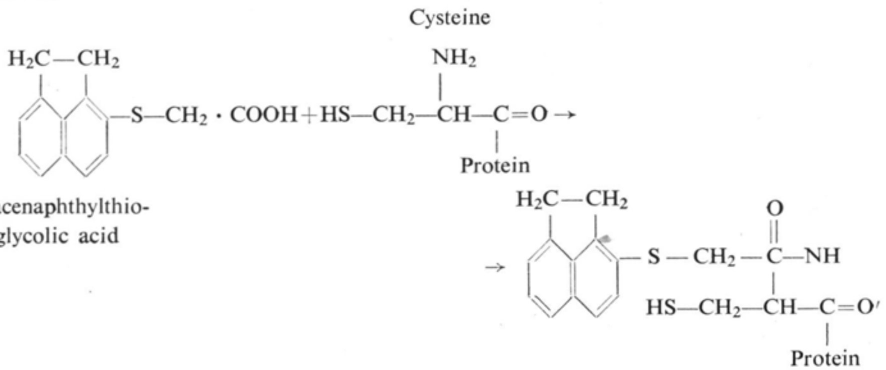
with acenaphthylmercaptoalkanocarboxylic acids were much longer than the controls as early as on the second day following germination.

It results from table 3 that the length and fresh weight of lettuce seedlings grown in water hardly differ from the corresponding values for lettuce seedlings treated with acenaphthene. The length and fresh weight of lettuce seedlings with acenaphthylmercaptoalkanocarboxylic acids differ from the corresponding values obtained for control seedlings. The differences are statistically confirmed. It is worth stressing that the lowest increase in the fresh weight of lettuce roots was found in seedlings treated with 3-acenaphthylthioglycolic acid, while the medium and highest increase in fresh weight were found in seedlings treated with 3-acenaphthylthio-(α-methyl)-

-glycolic acid and 3-acenaphthylthio-(α-ethyl)-glycolic acid, respectively. It should be stressed that the average values of fresh weight of hypocotyls of control seedlings (growth in water and those treated with acenaphthene) do not differ much when compared with the corresponding values concerning the hypocotyls of lettuce seedlings grown in the presence of the acids in question.

The results obtained suggest a relationship between the promotion of root growth, on the one hand, and the size of the side carbon chain of the acids applied, on the other. The presence of sulphur atom in the side carbon chain of the acids in question is supposed to play an important role in the metabolic changes in the plant organism. At present, however, this suggestion is a hypothesis only and must be confirmed by experiment.

The present stage of investigations permits only to accept the following interpretation of the activity of acenaphthylmercaptoalkanocarboxaylic acids in the living plant cell: the agents in question become physiologically active when combined with plant protein, for instance, through cysteine according to the following scheme.



3-acenaphthylthio-
glycolic acid

Active form

The scheme presented refers to the hypothesis advanced by Muir and Hansch (1953) who explained the physiological activity exerted by some known growth substances. This hypothesis is not in disagreement with that expressed by Marré and Arrigoni (1957) who are of the opinion that all substances containing —SH groups, used in low concentrations, counteract the growth-inhibiting substances. And thus the conclusion may be drawn that acenaphthene itself competitively inactivates native sulphydryl groups in the cells of actively growing parts of plant organs. As has been demonstrated, the acids examined by us showed no growth-inhibiting properties, although the acenaphthene system is a part of their chemical composition. The protective role against this activity might be ascribed to the side carbon chain including sulphur. The results obtained encourage to further investigations in order to elucidate to what degree our hypothesis with regard to the physiological activity of acenaphthylmercaptoalkanocarboxylic acids in plant organism will prove valid.

Our thanks are due to Professor Janczewski, head of the Department of Organic Chemistry, M. Curie-Skłodowska University, Lublin for encouragement to write this paper.

CONCLUSION

1. Acenaphthene showed no considerable effect on the germination of seeds and growth of roots of lettuce seedlings.

2. The derivatives of acenaphthene: 3-acenaphthylthioglycolic acid, 3-acenaphthylthio-(α-methyl)-glycolic and 3-acenaphthylthio-(α-ethyl)-glycolic acids stimulate dark germination of seeds and growth of roots of lettuce seedlings.

3. Short red-light irradiation of seeds saturated with the examined acids enhances the energy of germination.

4. A relationship was found between the size of the side carbon chain of the acids examined and their ability of promoting germination of seeds and of stimulating growth of roots of lettuce seedlings.

5. The results concerning the effect of promoting seed germination and growth of root seedlings of lettuce prove that the acids studied exhibit properties of growth substances.

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Kwasy acenaftylomerkaptoalkanokarboksylowe jako substancje wzrostowe roślin

Streszczenie

Badano wpływ acenaftenu i jego pochodnych o charakterze kwasów acenaftylomerkaptoalkanokarboksylowych na kiełkowanie nasion i wzrost siewek sałaty (*Lactuca sativa* L., odmiana Böttnera, partia 306).

Acenaften w stężeniu 0,01 mg/ml nie wpływał na kiełkowanie nasion i wzrost korzeni ośmiodniowych siewek sałaty w warunkach całkowitej ciemności. Kwasy natomiast przeciwnie, w tych samych stężeniach i warunkach stymulowały te procesy.

Naświetlanie czerwienią o długości fali 630 mμ w ciągu 5 minut wyraźnie podwyższało energię kiełkowania nasion sałaty nasyconych badanymi kwasami. Stwierdzono zależność między wielkością bocznego łańcucha węglowego badanych kwasów a ich zdolnością stymulowania kiełkowania nasion i wzrostu korzeni siewek sałaty.

W oparciu o wyniki przedyskutowano możliwość przybierania aktywności fizjologicznej kwasów acenaftylomerkaptoalkanokarboksylowych dzięki występowania bocznego łańcucha węglowego z obecną w nim siarką.

Otrzymane wyniki stymulowania kiełkowania nasion i wzrostu korzeni siewek sałaty świadczą, że badane kwasy wykazują właściwości substancji wzrostowych.