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STUDY IN THE LABORATORY OF THE ONE-STAGE WATER EXTRACTION OF SUGARS FROM KORINTHIAN RAISINS

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Summary

The one-stage water extraction of sugars from Korinthian raisins was studied experimentally for the determination of the optimum conditions to be applied. It was found that for obtaining the best results from this extraction it was necessary to keep certain conditions concerning:

- the currants weight to water volume ratio,
- the extraction temperature, and
- the extraction time (duration).

The optimum extraction conditions were accomplished by determining the sugars as well as the nitrogen compounds in the extract.

By keeping constant one parameter each time the other two were studied. The obtained results are presented in Table 4 and in Figures, 3, 4 and 5.

The changes observed above the optimum conditions are subject to discussion.

In the case of extraction of nitrogen compounds, the currants weight to water volume ratio was kept constant in all series of experiments, viz. 1:5, while the temperature changed. The extraction time varied while the temperature was kept constant for each experiment. The results obtained are presented in Figures 6 and 7.

Key words: Korinthian raisins, One-stage water extraction, Currants, Extracted sugars, Optimum extraction temperature and time, Extracted nitrogen compounds.

Introduction

Customarily, part of the annual produce of currants in Greece (Korinthian raisins variety), although edible, is not consumed as such, but is rather used as raw material for the production of sugar extracts, alcohol, raisin syrups and vinegar. In this way the producers succeed in making useful the whole amount of their product.¹

None of the other raisin producer countries (U.S.A., Australia, etc) face such a problem, since their product is consumed as edible in local and international markets, but in Greece the problem remains vital for the producers and the national economy. It is reported that more than 40% of the 1971 currant produce was industrialized and about 58% was exported abroad as edible.^{2,3}

According to 1966 statistical documents, Greece is at the top of the Korinthian raisins world production covering 84% of the total.⁴

In spite of the significance of the problem, and as far as we know, no systematic work on water extraction of sugars from currants has appeared in local or international bibliography.

Composition of Currants

It should be noted that currant composition is influenced by many factors such as soil, cultivation techniques, climatic conditions, etc.

The average composition of Korinthian raisins is given⁵ in Table 1.

TABLE 1. *Composition of Greek Korinthian raisins.*

	Percentage on natural currant basis	Percentage on dry currant basis
Acidity in sulfuric acid	1,305	1,591
Acidity in tartaric acid	1,997	2,434
Sugar	66,1	80,58
Tannic substances	0,757	0,923
Total ash	1,892	2,307
Extracted solid matter	75,30	91,79
Insoluble matter	6,739	8,215
Water content	17,961	—
pH (10g of dry currants in 1 lit of water)	3,65	—

TABLE 2. *Results of chemical analysis of Korinthian raisins used.*

	Percentage content	Method used ¹⁴
Reducing sugars	71,0*	LANE-EYNON
Water content	15,0	Conventional
Ash	1,62	Classic
Nitrogen compounds	0,42	KJELDAHL KJELDAHL-MATVEEF

*In Tables 2, 3, 4, 5, 6, and 7 all values are means of five replications.

It has been found, after six hours extraction, that the average content of currants in water insoluble materials is 4,86-7,97%, in potassium 0,708-0,95%, in calcium 0,05-0,078%⁶ and in proteins 2,3-2,6%^{7,8}. It has also been found that during drying vitamins A and C are destroyed, while vitamins B are not affected.⁶

As far as the sugars are concerned it can be said that they consist mainly of fructose and glucose with a ratio of about 0,92⁹. Pectin substances, contained in Korinthian currants, i.e. polygalactouronic acid products, are about 0,85%¹⁰ and they are found in their cell walls.^{6,11,12} The amount of the tannic substances present has been found to be 0,3-2%, it has also been found that they are constituents of the currant skin.¹³

With regard to proteins, it is reported¹⁴ that during denaturation, breaking off of their cross bonds takes place, which results in free rotation of the protein molecule, and the polypeptidic chains are unrolling and unfolding.

Experimental work

Particle size and chemical analysis of currants

For the experiments of the present work currants of 1972 harvest were used.

The mean diameter of the currants was determined granulometrically by screening,¹⁵ and the result taken was 7,5 mm.

The findings from the quantitative analysis of the currants for reducing sugars, water content, ash and nitrogen compounds are presented in Table 2.

Extraction apparatus

The extraction experiments were carried out by using the apparatus shown in Figure 1.

It consists of a 1 lit double wall glass flask E, into which the currants are placed. For the temperature regulation of the extraction experiments water is circulated by a thermostat from A to B. F, C and D are thermometer, pipe for the addition of the extraction water, and extract outlet pipe, respectively.

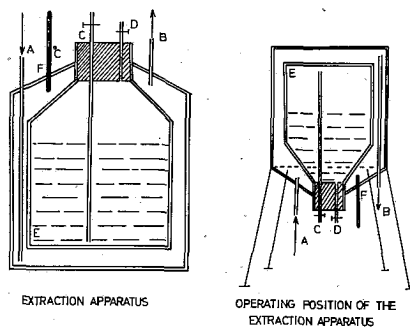


FIG. 1

Determination of optimum extraction conditions

In order to determine the optimum extraction conditions, i.e. a) the optimum currants weight water-volume ratio, b) the optimum extraction temperature and c) the optimum extraction time (duration), first the optimum currants weight water-volume ratio was determined and then keeping constant this ratio the determination of b) and c) optimum followed.

Determination of optimum currants weight water-volume ratio

In determining this ratio 100 g of currants were extracted at 71°C for 1 hour, while the volume of water used was varied. The results are presented in Table 3.

Plotting of the amount of extracted sugars against the used volume of extraction water is given in Figure 2.

It can be seen (Table 3) that for 100 g of currants, if the amount of extraction water from 100 ml increases the amount of extracted sugars also increases till the volume of extraction water reaches the value of about 500 ml. A farther increase of the amount of the extraction water beyond this value has no significant effect on the extracted amount of sugars.

Determination of optimum extraction temperature and time

For the determination of the optimum extraction temperature and time in all the experiments 100 g of currants and 500 ml of extraction water were used. For each series of experiments the temperature was kept constant but the time of the extraction varied.

The results are given in Table 4:

Plottings (a) of the amount of extracted sugars against extraction time at constant extraction temperature, (b) of the amount of extracted sugars against extraction temperature at constant extraction time and (c) of the extraction time against extraction temperature at constant amount of extracted sugars, are given in Figures 3,4 and 5, respectively.

TABLE 3. *Effect of extraction water volume on the amount of extracted sugars.*

Extraction water volume (ml)	Extracted sugars, g
110	14,76 a*
150	18,84 b
200	21,20 c
300	23,27 d
400	24,32 e
500	24,69 f
600	24,73 f
700	24,90 f
800	25,18 g

*Means in a column followed by the same letter are not different at the 1% level of significance.

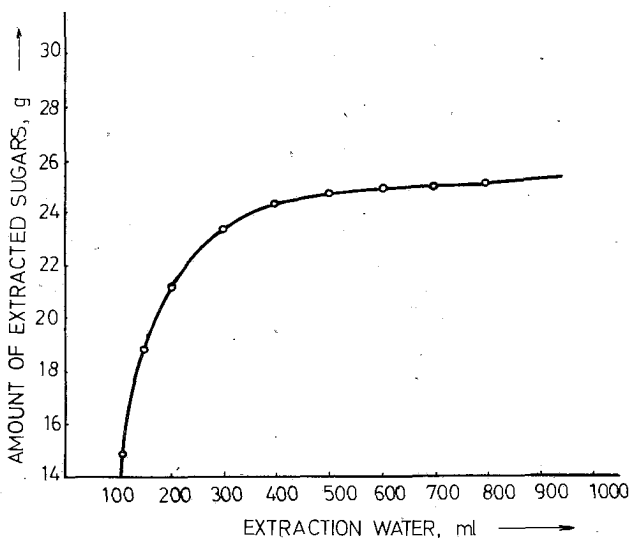


FIG. 2: *Amount of extracted sugars in relation to extraction water volume at 71°C temperature for 1 hour extraction time.*

TABLE 4. Effect of the extraction duration on the amount of extracted sugars at various extraction temperatures.

Time (hr)	Quantity of extracted sugars at various temperatures (g)														
	25°C	35°C	45°C	50°C	57°C	60°C	65°C	71°C	75°C	78°C	80°C	83°C	86°C	90°C	93,5°C
1/6	7,21 a	8,61	9,02	9,42	9,77	10,01	10,40	10,61 a	11,22	11,61	12,07	15,81 a	16,32	16,43	16,51 a
1/3	8,52*	10,32	10,78	11,00	11,10	11,21	11,86	12,52	15,31	17,00	18,03	19,00	20,08	21,31	22,36
1/2	10,95 b	12,65	12,98	13,02	14,01	15,32	18,23	20,00 b	21,20	22,70	23,06	23,87 b	24,41	24,80	25,02 b
1	16,79 c	18,07	18,30	18,51	19,02	20,00	22,87	24,69 c	26,94	27,03	27,72	28,93 c	29,10	29,51	29,62 c
2	22,21	23,96	26,12	28,40	29,08	30,07	31,22	32,30	32,52	32,60	33,12	33,30	33,43	33,76	34,17
3	26,20 d	28,64	31,42	34,21	35,01	37,53	37,98	38,33 d	36,21	35,63	37,51	37,70 d	37,92	38,00	38,27 d
4	29,49	32,01	35,93	37,61	40,42	42,51	42,57	42,62	40,31	38,84	42,27	40,49	42,32	43,00	42,47
5	32,40 e	36,00	39,13	40,72	43,81	46,51	47,41	50,78 e	43,10	41,02	43,29	42,27 e	44,61	44,71	46,01 e
6	34,72	38,67	42,44	44,82	47,23	50,03	51,04	54,16	45,03	42,40	44,02	43,91	45,31	45,50	49,02
7	37,20 f	41,00	47,12	47,90	50,21	53,00	53,92	57,51 f	47,21	43,60	45,51	44,50 f	45,62	46,00	51,51 f
8	38,90	43,26	48,01	50,43	52,00	55,50	56,15	58,00	48,73	44,46	46,00	44,68	45,67	46,43	53,02
9	40,90 g	45,29	50,20	52,51	54,20	57,51	58,41	59,09 g	50,50	44,92	46,12	44,70 f	45,72	46,71	56,01 g
10	42,51	47,91	52,21	54,30	55,71	59,02	59,80	60,05	52,00	45,60	46,20	44,76	45,83	46,83	57,42
12	46,01 h	50,03	55,46	57,31	58,41	61,71	61,89	62,02 h	54,72	46,61	46,50	44,81 f	45,89	47,12	60,12 h
15	49,03 i	53,01	57,81	59,22	60,40	62,51	62,60	62,80 h	57,60	47,20	46,50	45,00 f	46,00	47,20	62,00 i
20	53,10 j	57,09	59,00	60,20	61,85	63,75	64,77	64,91 i	60,09	47,57	46,51	45,03 f	46,02	47,22	63,17 i
30	53,12 j	57,12	59,01	60,61	62,95	64,21	64,79	65,01 i	60,02	47,59	46,61	45,01 f	46,02	47,21	64,02 i
40	53,17 j	57,10	59,05	60,79	63,05	64,51	64,81	65,01 i	60,07	47,60	46,61	45,03 f	46,02	47,23	64,00 i
45	53,17	57,13	59,07	60,79	63,07	64,50	64,81	65,01	60,07	47,60	46,60	45,04	46,01	47,22	64,06

*Means not followed by a letter have not been analysed statistically (Tables 5, 6 and 7).

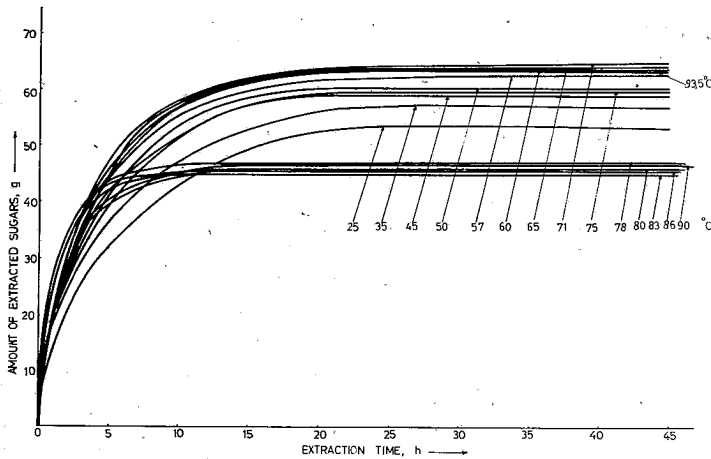


FIG. 3: *Extraction isotherms*

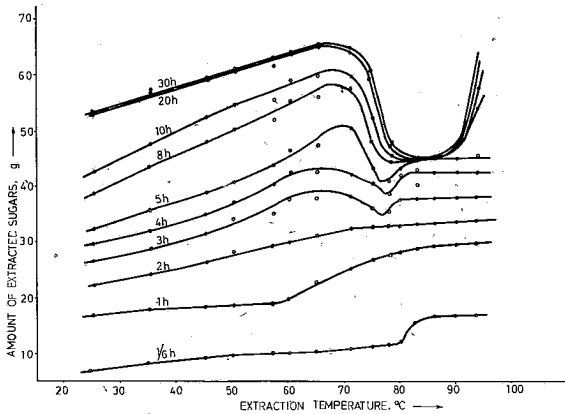


FIG. 4: *Extraction isochronous*

As can be seen from Table 4, Figure 3, in the case of constant temperature extraction the amount of extracted sugars increased with extraction time up to a value, above which there was no significant change. It may be said that in this time the extraction process reached the state of equilibrium. The required time for this equilibrium to be reached was about 20 hours for the extraction temperatures of 25, 35, 45, 50, 57, 60, 65, 71 and 75°C.

Between the temperatures of 75°C and 86°C the time required for this equilibrium to be reached decreased: it was approximately 15, 8, 7 and 6 hours for the temperatures of 78, 80, 83 and 86°C, respectively.

At the temperatures of 90°C and 93,5°C it was observed that the time required for the equilibrium to be reached increased again, and it was equal to 8 and 30 hours, respectively.

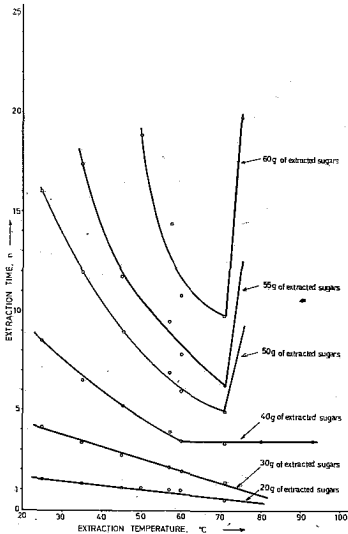


FIG. 5: *Extraction isobars.*

Under constant extraction time and up to 2 hours the amount extracted sugars increased by increasing the temperature from 25 to about 93,5°C (Fig. 4). From 2 to 45 hours extraction time the amount of extracted sugars increased by increasing the temperature up to about 71°C, then it decreased from 75 to about 83°C and finally it increased again from 86 up to about 93,5°C.

Determination of extracted nitrogen compounds

For this determination experiments were carried out at constant extraction temperature within the range of 25 to 93,5°C. The extraction time was for all experiments constant and equal to 25 hours. The currants weight water-volume ratio was in all cases equal to 1:5. The results are given in Table 5.

Plotting of the amount of extracted nitrogen compounds against extraction temperature is given in Figure 6.

Extraction experiments were also carried out under constant extraction time equal to 1, 5, and 25 hours at temperatures of 50, 71, 83 and 93,5°C each time.

The results from the extraction experiments are given in Table 6.

Plotting of the amount of extracted nitrogen compounds against extraction temperature is given in Figure 7.

The results of the nitrogen compounds extraction experiments showed that:

1) For 25 hours extraction time an increase in the extraction temperature from 25 up to 50°C was followed by an increase in the amount of extracted nitrogen compounds (Table 5). Then for the extraction temperature region above 50 and up to 71°C the extracted amount of nitrogen compounds gradually decreased. By increasing the temperature above 71 and up to 83°C the amount of extracted nitrogen compounds was increased again, and, finally, for extraction temperature higher than 83 and up to about 93,5°C the amount of extracted nitrogen compounds was again decreased.

TABLE 5. *Effect of the extraction temperature on the amount of extracted nitrogen compounds.*

Extraction temperature °C	Amount of extracted Nitrogen compounds (mg N)	Extraction temperature °C	Amount of extracted Nitrogen compounds (mg N)
25	88,0 a	75	107,6
35	97,8	78	113,8
40	102,0	80	119,2
50	110,4	83	124,4 d
55	109,5	85	115,3
60	108,8	90	103,6
65	107,8	93,5	92,2 b
71	107,0 c		

TABLE 6. *Effect of the extraction temperature at various extraction durations on the amount of extracted nitrogen compounds.*

Extraction temperature °C	Amount of extracted Nitrogen compounds in 1 hour, mg N	Amount of extracted Nitrogen compounds in 5 hours, mg N	Amount of extracted Nitrogen compounds in 25 hours, mg N
50	48,8 a	98,4 b	110,4 c
71	56,0 b	103,4 c	107,0 b
83	70,0 c	106,2 d	124,4 d
93,5	70,1 c	86,6 a	92,2 a

TABLE 7. *Effect of extraction temperature on the pH values of after-extraction-currants pulp.*

Extraction temperature °C	pH
25	3,56
71	3,31
83	3,15
93,5	3,02

2) In the case of extraction under constant temperature, equal to 50, 71, 83 and 93,5°C in each series of experiments and for extraction time 1,5 and 25 hours, the amount of extracted nitrogen compounds was increased with temperature (Table 6).

The difference of the amount of extracted nitrogen compounds between 1 and 5 hours extraction decreased as the extraction temperature increased. Generally a similar pattern was also observed for the amount of extracted nitrogen compounds difference between 1 and 25 hours extraction. This difference was almost the same for the temperatures of 71 and 83°C.

3) In one hour extraction experiments, there was observed a gradual increase in the amount of extracted nitrogen compounds as the temperature increased from 50 to 71 and then to 83°C, while the corresponding value at 93,5°C was about the same with that received at 83°C.

4) Under constant extraction time equal to 5 and 25 hours, in each case, an increase of the amount of extracted nitrogen compounds took place until extraction temperature reached 83°C and then a decrease followed the temperature of 93,5°C.

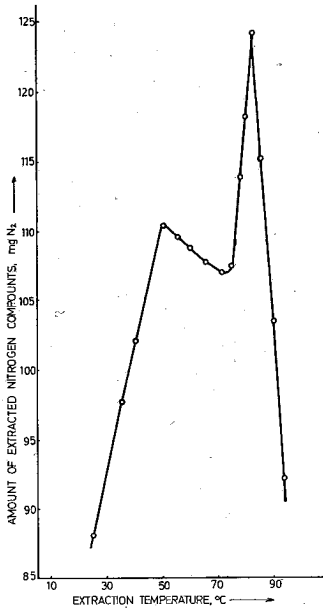


FIG. 6: Amount of extracted Nitrogen compounds for 25 hours extraction time in relation to temperature.

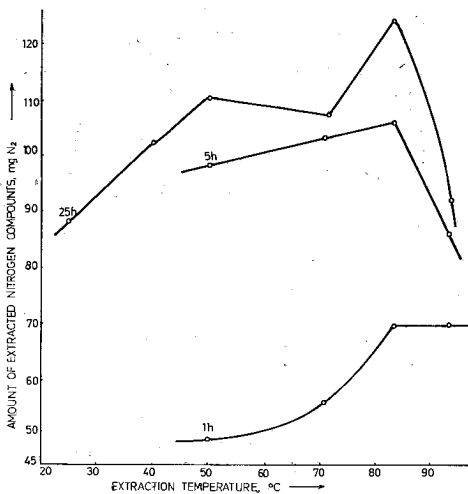


FIG. 7: Isochronous of amount of extracted Nitrogen compounds in relation to temperature.

5) The plottings of the amount of extracted nitrogen compounds and sugars, for 25 hours extraction, against extraction temperature (Figure 8) showed that at temperatures 71, 83 and 93,5°C the amount of extracted sugars was maximum, minimum, and maximum, respectively, though the amount of extracted nitrogen compounds was minimum, maximum, and minimum, i.e. when the amount of extracted sugars was maximum, the amount of extracted nitrogen compounds was minimum, and vice versa.

After-extraction-currants pulp pH change in relation to extraction temperature

The pH measurements were carried out by using a METROHM E396B pH-meter with compound calomel-glass electrode type EA120X. The pulp used was produced by pulping the currants which had been extracted for 25 hours at different extraction temperatures. The observed pH values for 25, 71, 83, and 93,5°C are given in Table 7.

Plotting of extraction temperature against pH is given in Figure 9.

Discussion

The results of this experimental work are as follows:

1) In the case of constant time extraction and up to 2 hours the amount of extracted sugars increased when temperature increased from 25 to about 93,5°C (Table 4). This may be due to the following reasons:

a) To the increase of the denaturation velocity of the protoplasmatic proteins as the extraction temperature increased. A consequence of this fact is that diffusion is enhanced as in the case of sugarbeets.¹⁶

b) To the increase of the mobility of sugar molecules with increasing temperature, and

c) To the expansion of cell wall and currants skin pores. A variety of other factors may also be at work here.

2) In the case of constant extraction time higher than 2 hours the amount of extracted sugars increased when the temperature increased from 25 to 71°C. This fact also may be due to the reasons mentioned above.

3) In the case of constant extraction time higher than 2 and not greater than 45 hours a decrease in the amount of extracted sugars was observed as the temperature increased from 71 to about 83°C then a further increase in temperature up to about 93,5°C resulted again in an increase in the amount of extracted sugars.

The decrease of the amount of extracted sugars in extraction temperatures from 71 to about 83°C is probably caused by:

a) The inhibition of the diffusion of sugars because of the differentiation of structure of the protoplasmatic proteins, which takes place at long heating periods at these temperatures.¹⁷

b) The jellyfication of sugars, caused by the presence of pectins, under proper pH values of cell juice.¹⁸

c) The loss of inflexibility of cell membranes and skin of currants, which is acceptable for sugarbeets.¹⁹ There may also be other reasons determining this decrease.

As far as the amount of extracted nitrogen compounds is concerned the experimental results are as follows:

25 hours extraction

1) When extraction temperature increased from about 25 to 50°C, an increase in the amount of extracted nitrogen compounds was noted which is probably due to the increase of mobility of protein molecules with temperature. This phenomenon seems to rule over all other factors in this case, because the protein denaturation, which inhibits the extraction, takes place at small velocity for these temperatures.¹⁹

2) When extraction temperature increased from about 50 to 71°C the amount of extracted nitrogen compounds decreased. This may be due to the velocity increase of protein denaturation with temperature.¹⁹ This phenomenon may be

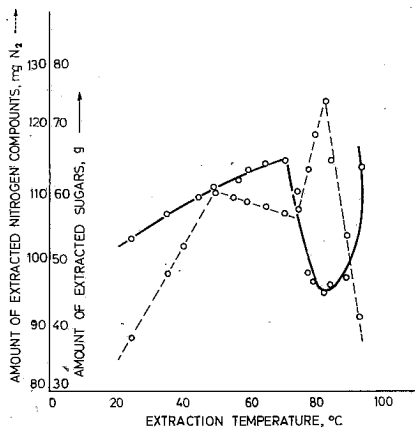


FIG. 8: Comparison of the amounts of extracted sugars and nitrogen compounds.

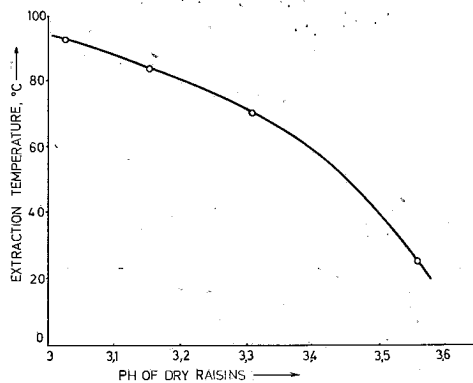


FIG. 9: pH change in relation to extraction temperature.

considered counterpoise to and of greater magnitude than the increase of protein mobility. If this is true the result is the gradual decrease of the amount of extracted nitrogen compounds in this temperature range.

3) When extraction temperature increased from about 71 to 83°C an increase in the amount of extracted nitrogen compounds was again observed.

The following probable causes may be considered in attempting an explanation of this phenomenon.

a) The increase of protein molecules mobility which rules over the denaturation effect.

b) The change of protein molecules shape.

c) The enlargement of cell walls and currant skin pores.

It may be said that these phenomena take place before the loss of inflexibility and blocking of cell walls and currant skin, because of jellyfication which is caused by sugars, in presence of pectin substances under favorable pH value of currant cell juice.

4) When the extraction temperature increased from about 83 to 93,5°C the amount of extracted nitrogen compounds decreased again. This is probably due to complete coagulation of proteins, because the large micelles that are formed cannot pass through cell walls and skin of currants.

Conclusions

1) In the case of extraction temperature 71°C, for 1 hour, and with constant amount of currants, the amount of extracted sugars increased with the amount of extraction water, till the ratio of currant weight to water-volume reached the value 1:5. When the value of this ratio decreased the amount of extracted sugars did not change significantly. Therefore it may be concluded that the optimum currants weight to extraction water-volume-ratio under the mentioned extraction conditions is about 1:5.

2) Working with 1:5 currants weight to extraction water volume ratio and under constant temperatures the amount of extracted sugars increased with extraction time. The amount of extracted sugars reached a maximum, depending on temperature, after which a further increase in extraction time resulted in no significant change in the amount of extracted sugars. The extraction time for maximum amount of extracted sugars to be achieved depended also on temperature, and for 71°C it was about 20 hours.

3) The amount of extracted sugars from 100 g currants under 20 hours extraction time and temperatures of about 50, 57, 60, 65, 71 and 93,5°C was 60.20, 61.85, 63.75, 64.77, 64.91, and 63.17 g, respectively. Since the total sugar content of the currant samples used was 71%, the corresponding percentages of these amounts are 84.71, 87.11, 89.80, 91.23, 91.42, and 88.97.

The amount of extracted sugars from 100 g of currants under 30 hours extraction time and at 93,5°C was 64.02 g, that is 90.17% of the total amount of sugars.

Generally it may be concluded that the maximum amount of sugars that can be extracted by using only one extraction may be achieved by observing the following optimum extraction conditions:

- a) Currant weight to water volume ratio: 1:5.
- b) Extraction temperature: Between 60 to 70°C.
- c) Extraction time: About 20 hours.

4) With respect to the amount of nitrogen compounds co-extracted with sugars at 25 hours extraction time and at temperature of 71, 83, and 93,5°C, it was found that the maximum values of nitrogen compounds correspond to minimum ones of sugars, and vice versa.

Περίληψη

Έργαστηριακή μελέτη άπλης μέ νερό έκχυλίσεως τών σακχάρων από τήν κορινθιακή σταφίδα

Στήν έργασία αύτή γίνεται έργαστηριακή πειραματική μελέτη για τόν προσδιορισμό τών βέλτιστων συνθηκών άπολήψεως τών σακχάρων από τήν

κορινθιακή σταφίδα με μιὰ μόνο ἐκχύλιση με νερό. Στην ἀρχὴ βρέθηκε ὁ βέλτιστος λόγος βάρους σταφίδας/ὄγκου νεροῦ. Με σταθερὴ αὐτὴ τὴν παράμετρο προσδιορίστηκαν πειραματικὰ ἡ βέλτιστη θερμοκρασία καὶ ἡ βέλτιστη διάρκεια τῆς ἐκχυλίσεως με κριτήριον τὴν ποσότητα τῶν παραλαμβανομένων σακχάρων. Παράλληλα ἔγιναν προσδιορισμοὶ καὶ τοῦ ὀλικοῦ ἀζώτου στὸ ἐκχύλισμα.

Τὸ συμπέρασμα ποὺ προκύπτει εἶναι ὅτι γιὰ νὰ παραληφθεῖ ἡ μέγιστη δυνατὴ ποσότητα σακχάρων ἀπὸ τὴν κορινθιακὴ σταφίδα με μιὰ μόνο ἐκχύλιση πρέπει νὰ τηρηθοῦν οἱ ἀκόλουθες συνθήκες:

- 1) Βάρος σταφίδας πρὸς ὄγκο νεροῦ ἐκχυλίσεως: 1:5.
- 2) Θερμοκρασία ἐκχυλίσεως: Μεταξὺ 60 καὶ 70°C.
- 3) Διάρκεια ἐκχυλίσεως: 20 ὥρες περίπου.

Σὲ ὅ,τι ἀφορᾷ τὴν ποσότητα τῶν συνεκχυλιζομένων με τὰ σάκχαρα ἀπὸ τὴ σταφίδα ἀζωτούχων οὐσιῶν παρατηρήθηκε ὅτι με διάρκεια ἐκχυλίσεως 25 ὥρες γιὰ τὶς θερμοκρασίες 71, 83 καὶ 93,5°C οἱ μεγαλύτερες τιμὲς σακχάρων στὸ ἐκχύλισμα ἀντιστοιχοῦν σὲ μικρότερες τιμὲς ἀζωτούχων οὐσιῶν καὶ ἀντίστροφα.

Τέλος, γιὰ κάθε πειραματικὴ παρατήρηση δίνεται καὶ ἡ πρὸ πιθανῆ αἰτιολογία.

Ὁρολογία: Κορινθιακὴ σταφίδα, Μία μόνο ἐκχύλιση με νερό, Σταφίδες, Ἐκχυλιζόμενα σάκχαρα, Βέλτιστη θερμοκρασία καὶ διάρκεια ἐκχυλίσεως, Ἐκχυλιζόμενες ἀζωτούχες οὐσίες.

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STUDIES ON THE CHEMISTRY OF HUMIC ACIDS II. INTERACTION OF Fe(III) AND Cu(II) WITH OXYGEN CONTAINING POLYMERIC LIGANDS.

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Summary

The investigation of the interaction of Fe(III) and Cu(II) with toluoquinone and oligomeric products of its oxidative condensation¹ is extended here to more complicated polymeric species, having a closer resemblance to the natural humic acids. This resemblance is reflected in the electronic, vibrational and ESR spectra, as well as in some properties relevant to their function in nature, such as the acidity, complex formation capacity etc, and is attributed to specific similarities in structure.

Key words: Humic acids, oligomeric and polymeric species, oxidative condensation, complexes.

Introduction

The general objectives of the investigation have been formulated in the first paper of this series¹ (Part I) where we reported our findings on the interaction of Fe(II), Fe(III) and Cu(II) with toluoquinone and its oligomeric products of oxidative condensation. These products are known components in the soil.^{2,3} In this paper we extend the investigation to more complicated polymeric ligands. In the line connecting the two extremes, namely the natural systems (soil, natural waters, sediments etc.) on the one hand and "pure" chemistry on the other, Part I lies closer to "pure" chemistry, whereas Part II lies closer to the natural end of the line. This means that in Part II the restrictions imposed by the system are more severe and hence the treatment is inherently less vigorous.

Experimental

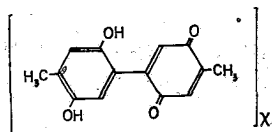
"Artificial" humic acid. The artificial humic acid* used in this work is obtained by the spontaneous condensation of toluoquinone in acid solutions.

Three to five ml of concentrated H₂SO₄ are added to 100 ml of a saturated solution of toluoquinone, containing ca 2g toluoquinone. The mixture is condensed at low temperature to ca 25 ml. The dark-gray precipitate formed is filtered, washed with cold water to remove SO₄²⁻, toluoquinone and toluohydroquinone, and is dried under vacuum.

* Abbreviated AHA. The component of humic substance of a natural source (soil, lake deposits etc.) which is soluble in dilute alkali but insoluble in acid, will be abbreviated NHA (Natural Humic Acid) and the Component of humic substance of a natural source which is soluble both in dilute alkali and acid will be abbreviated NFA (Natural Fulvic Acid).

Typical analysis of the product

C 67.77, H 4.55, O 27.47 (oxygen from the difference) corresponds fairly well to the formula



(C 68.85, H 4.91, O 26.23)

The concentration of the acid vary from 0.5 to 9 N and instead of sulphuric, hydrochloric or phosphoric acids can be used. The same product is also apparently obtained from the oxidative condensation of toluhydroquinone in alkaline solutions.^{4,5} The condensation of hydroquinones in acid solution is generally more difficult.

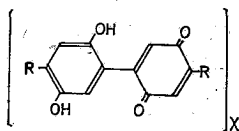
Polymerization products analogous to those obtained from toluoquinone and by an analogous procedure were also obtained from simple o- and p- benzoquinone and from ethylquinone (ethyl-p-benzoquinone) The analyses gave:

polymer from o-benzoquinone C 62.65, H 4.78 O 32.57

polymer from p-benzoquinone C 66.01, H 4.56 O 29.43

polymer from ethylquinone C 70.69, H 5.74 O 23.57

The corresponding calculated values on the basis of the general formula:



for R = H C 66.05, H 4.62, O 29.35

for R = C₂H₅ C 69.56, H 7.24, O 23.18

Mixtures of AHA with copper and iron salts. Mixtures containing AHA and Cu(II) or Fe(III) in various initial ratios were prepared as follows:

- Copper (II) nitrate (Cu(NO₃)₂·3 H₂O) was added to a suspension of AHA in water or dilute alkali. The precipitate was filtered, washed with water and dried under vacuum at 80°C. Its color varied from dark green to brown, depending on the composition.
- The metal salt [Cu(NO₃)₂·H₂O or Fe(NO₃)₃·5H₂O] was added to a homogeneous solution of AHA in a water-methanol mixture (90:10). The solution was then condensed on a steam bath and the precipitate formed filtered, washed with water and dried under vacuum at 80°C.
- The acid (H₂SO₄) condensation of toluoquinone was done in the presence of Cu(II) or Fe(III).

The analysis of the various mixtures depends on the initial amounts of the reagents used and on the method of preparation. Typical compositions together with the IR spectra etc are given in the results (*vide infra*).

Titrations: Aqueous solutions of AHA (0.5-5%) are acidic (pH — 4.3) and with 0.1N NaOH they give titration curves with one dominant break.

Total and phenolic acidity were determined following the methods developed by Blom, Edelhausen and Van Krevelen⁶ and by Brooks and Starnhell⁷ as modified

by Schnitzer and Gupta.⁸ In the first of these methods the sample is treated with calcium acetate and the released hydrogen ions are titrated potentiometrically (glass-calomel electrodes) with 0.1N NaOH to PH 9.8. In the second method the sample is treated with barium hydroxide and the excess base is titrated with 0.5M HCl to pH 8.4. In the blank experiments the solutions did not contain AHA but the procedure followed otherwise was exactly the same to that followed for the samples.

The calcium acetate method presumably measures the acidity due to the carboxylic groups whilst the barium hydroxide method measures the total acidity. Phenolic acidity is obtained from the difference. Solutions of AHA containing known amounts of Cu(II) or Fe(III) were also titrated with 0.01N NaOH.

Instruments and Analysis. The same instruments were used for performing the elemental analysis and taking the electronic and vibrational spectra as in the previous paper of the series¹. ESR spectra of powder samples were taken with a Varian ESR spectrometer at a modulation frequency of 9055 GHZ. Spin content was estimated by comparison with diphenylpicrylhydrazyl.

Copper was determined, as previously, gravimetrically as CuSCN and/or CuO and electrolytically, and iron gravimetrically as Fe₂O₃.

Results

Acidity. The results of the titrations of the artificial humic acid are given in Table I. This table also contains values for typical soil humic acids, for comparison purposes.

The reliability of the "standard" methods used was assessed⁸ by applying them to a number of pure carboxylic acids (Table II). The curves of Fig. 1 were obtained by titrating with NaOH AHA solutions and AHA solutions containing Fe(III) or Cu(II). At relatively small metal ion to AHA ratios there are minor inflexion points but one break dominates (Fig. 1a); at larger metal ion to AHA ratios more breaks are clearly seen (Fig. 1b). The behavior is analogous to the behavior of natural humic acid⁹.

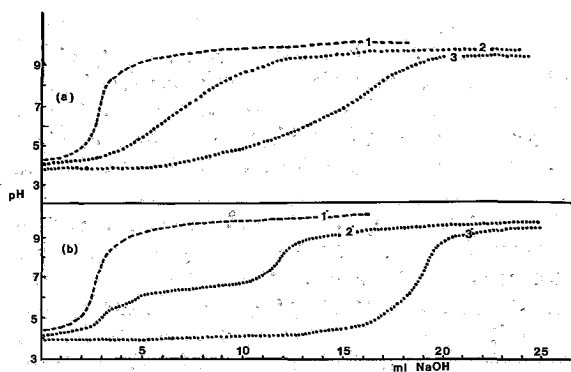


FIG. 1. Titration curves of solutions of AHA with 0.01M NaOH. All solutions contained 0.025 g of AHA dissolved in 250 ml of water (curves 1,1), 2ml Cu(NO₃)₂ 0.02 M (curve 2), 5 ml Cu(NO₃)₂ 0.01 M (curve 2), 2ml Fe(NO₃)₃ 0.01 M (curve 3) and 5 ml Fe(NO₃)₃ 0.01 M (curve 3)

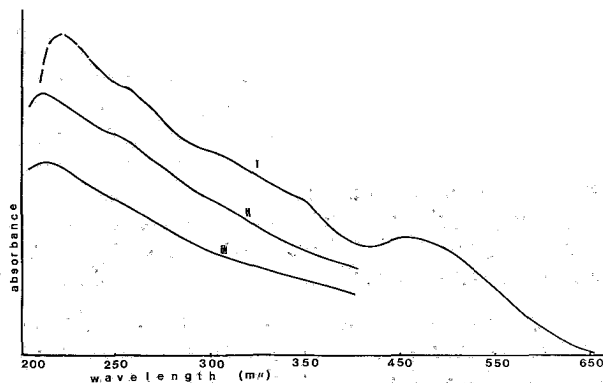


FIG. 2. Electronic spectra of I: AHA (this work), II: NHA (ref 10) and III: NFA (ref 10). Absorbance in arbitrary units.

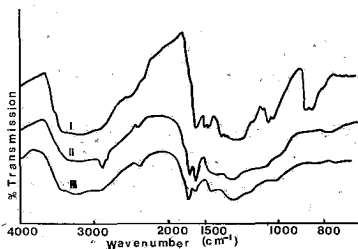


FIG. 3. Comparison of IR spectra of I AHA (this work), II NHA (ref 10) and III NFA (ref 10).

Spectra. UV and visible spectra of our artificial humic acid are compared in Fig. 2 to the spectra of natural humic and fulvic acids. The minimum for AHA at ca. 400 nm is essentially the onset of the strong absorption in the UV. The corresponding onsets of the absorption of NHA and NFA lie also in this region.

IR spectra of NHA and NFA¹⁰ and a typical spectrum of AHA are shown in Fig. 3.

Representative spectra of mixtures containing AHA and Fe (III) are given in Fig. 4. The corresponding initial compositions and analyses of the products are given in Table III. Fig. 4 includes spectra of mixtures of Fe (III) with natural fulvic acids.¹¹ Fig. 5 gives the IR spectra of the products obtained by condensation in the presence of copper sulphate (Table IV). These products are hard, resin-like and they show a characteristic sharp absorption in the UV around 340 nm (Fig. 6)

The spectra obtained by adding Cu(II) *after* condensation resemble very closely those of the Fe(III) mixture depicted in Fig. 4, but they are distinctly different from the corresponding spectra obtained by adding Cu(II) *before* condensation (Fig. 5).

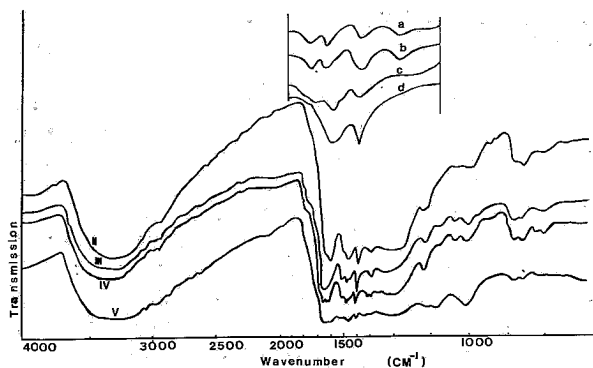


FIG. 4. IR spectra of mixtures containing Fe(III) and AHA or NHA. Ratios of Fe(III) over AHA (see Table III). II 1:2, III 2:2, IV 3:2, V 4:2. Ratios of Fe(III) over NFA (from ref 11): a without Fe(III), b 1:1, c 3:1, d 6:1.

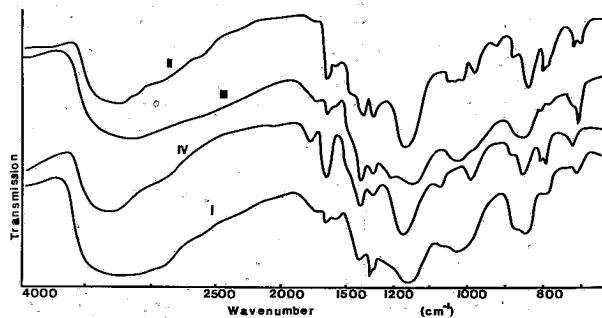


FIG. 5. IR spectra of the products of condensation of toluoquinone in the presence of Cu(II) (see Table IV). Ratios of Cu(II) over toluoquinone II 2:3, III 3:3, IV 6:3, (I blanc, without Cu(II)).

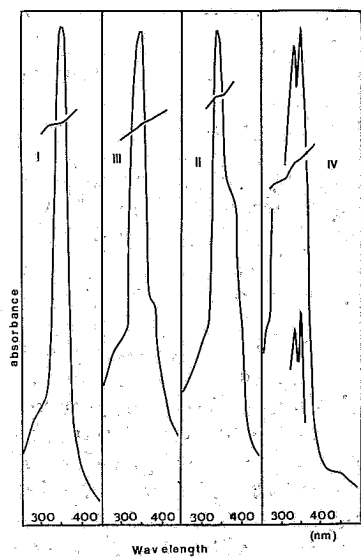


FIG. 6. Details of the electronic spectra of the product of condensation of toluoquinone in the presence of Cu(II) (Table IV).

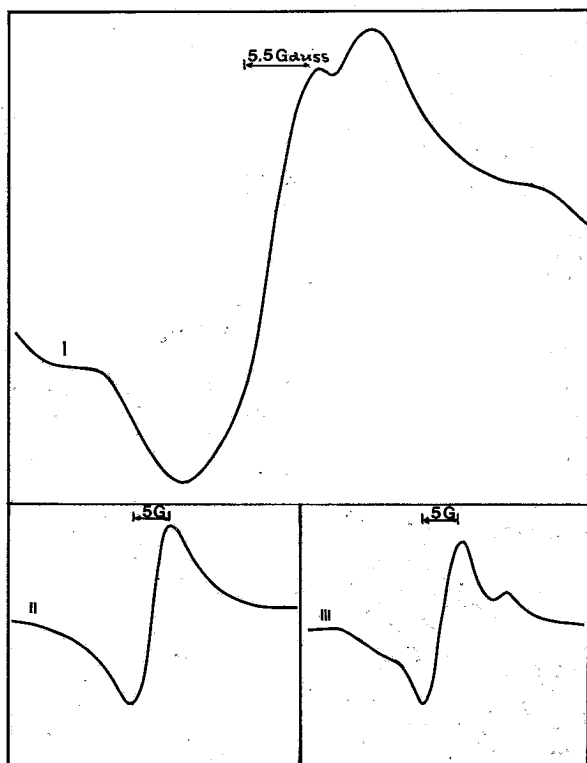


FIG. 7. Typical ESR powder spectra of: I AHA (this work), II Unhydrolyzed NHA from Switzerland (ref. 12) and III hydrolyzed NHA from England (ref. 12).

The ESR spectrum of AHA resembles also closely the analogous spectra of natural humic acids (Fig. 7) and spin concentrations are comparable¹² (Table V).

The g value corresponding to the inflection at 3219.5 G is 2.0100.

Discussion

It is important to point out from the outset that in a multicomponent system such as that studied here, direct and simple correlation of properties (e.g. spectra) and structure is virtually impossible. Yet, if we want the knowledge gathered not to remain in the form of uncorrelated fragments we must try to systematize it and since direct correlation between properties and structure is unattainable, the obvious alternative is indirect correlation. The method used here is based on a double comparison: to the simpler related systems (monomer, dimer, etc.) reported previously¹ and to the available data for natural products. Moreover, in making this comparison we have concentrated on the properties which are relevant to the

function of humic acids in the natural environment, and more specifically to the role they play in the ecology of the soil or natural waters.

The acidity data clearly show that the total number of acid groups obtained by the barium hydroxide method is a lower limit, whereas the number of carboxylic groups obtained by the calcium acetate method is an upper limit.

The deviation from theoretical values should mainly be determined by the number and the positions of the hydroxyl groups.

These conclusions are based on the data for monomeric carboxylic acids (Table II) and for our artificial humic acid (Table I) which does not contain carboxyl groups, but it is reasonable to extend them to natural humic acids as well.

The titration curves of humic acid-metal ion mixtures (Fig. 1), especially those obtained with excess metal ion, indicate fairly strong interaction persisting in solution. In the first paper of this series it has been concluded that the dimer and trimer of toluoquinone can form with metal ions polynuclear chelated complexes through properly positioned hydroxyl and carbonyl groups. The titration data suggest that this conclusion may be extended to the polymer. Moreover, the resemblance of the curves for artificial and natural humic acids is consistent with the assumption that natural humic acids can form real complexes as well. This resemblance appears even more intriguing if one recalls that our artificial humic acid differs from the natural products in the degree and/or the kind of condensation, the nature of the active polar groups, and the complexity. Yet, it seems that what is qualitatively more important in complex formation capacity, is the relative positions of the active groups.

Proper relative positions for chelate formation may be regarded as the common feature all humic acids, more or less, have and explains to a large extent the remarkable similarity in their function in the environment in spite of the many differences in their composition and origin. Polar groups (e.g. $-\text{OH}$, $-\text{C}=\text{C}$, $-\text{COO}^-$, $-\text{SO}_3\text{H}$, $-\text{NH}_2$ etc.) in places favoring chelation are expected to increase the capacity for complex formation. In the changes having this effect one can classify condensation (e.g. compare toluoquinone with its dimer or trimer¹) hydrolysis,^{13,14} aminolysis, sulfonation, oxidation etc.

In contrast, any change resulting in a decrease of neighboring active groups, such as furan ring formation, esterification, formation of species with active groups remote from each other etc., are generally expected to decrease complex formation capacity.

The understanding of the factors affecting complex formation capacity has also practical value. The issue at stake here is essentially understanding the way that humic acids participate in the mechanism of metal ion transport in the environment. More specifically it can help in selecting the kind of treatment natural sources of humic acids, such as lignite, should be subjected to, if we want to increase their effectiveness in this respect, and use them e.g. as fertilizers. It can also give ideas for the mobilization of trace and toxic metals through the sewage systems or the streams and their transport or immobilization in certain aquatic environments. The UV and visible spectrum of AHA is considerably red shifted and broader relative to the dimer and trimer¹ but strikingly similar to those of NHA and NFA (Fig. 2). The red shift and broadening can be attributed to the higher degree of condensation. The spectral similarity to the natural products can be attributed to a corresponding similarity in the kind and distribution of chromophores. The spectrum of AHA is obviously dominated by the strongly absorbing condensed benzene rings rather than the substituents, and that seems to be the case in the natural products too. The spectrum in this region is very insensitive to the usual substituents in humic acids.

Moreover, the overlap of the absorptions from the various condensation products present in the multicomponent system precludes any attempt to discern peaks and attribute them to individual species.

Yet, the overall shape of the spectrum still contains considerable information regarding the kind and distribution of strong absorbers.

The situation in the IR region is quite similar (Fig. 3). The spectra of AHA, NHA and NFA have a broad contour line instead of well resolved peaks but they are still useful: not because one can discern group frequencies but rather because they have absorptions in the regions expected on the basis of the spectra of the monomer, dimer and trimer. Moreover although we cannot talk about displacement of group frequencies upon complexation, we can still see (Figs 4 and 5) some gradual changes in shape, which do not prove complexation but are certainly consistent with it. We can also state that the products obtained by condensation in the presence of a metal ion (Fig. 5) are of a different nature than the products obtained by mixing the metal salt after acid polymerization (Fig. 4). This statement is further supported by the different physical appearance (hardness; colour, etc.) and the sharp peaks in the UV (Fig. 6) which are only observed with mixtures obtained by condensation in the presence of metal ion. In the condensation process the metal ion can act as a Lewis acid, but it probably also forms bridged polynuclear structures. In nature both condensation paths are in principle possible.

Finally, our artificial humic acid also resembles natural humic acids (especially the hydrolyzed ones) in its ESR spectrum (Fig. 7) and free radical content (Table V). The spectrum indicates low symmetry and/or contribution from two species. The high value of g can be attributed to the small extent of condensation.

TABLE I: Activity of Artificial and Natural Humic Acids

Substance	-COOH(meq.g ⁻¹)	Total acidity (meq g ⁻¹)	Ref.
<i>Humic acid</i>			
Gray wooded O-HA-1	3.0	8.7	9
Gray wooded O-HA-2	2.8	8.9	8
Podzolic O2-HA-1	2.2	5.7	16
Podzolic O2-HA-2	2.7	not determined	8
<i>Fulvic acid</i>			
Gray wooded O-FA	8.5	14.2	9
Gray wooded B2-FA	9.1	11.8	17
Podzolic Bh-FA	9.1	12.4	16
<i>Artificial Humic Acid</i>	3.4	8.45	*

* This work

TABLE II: *Acidity data for some aromatic carboxylic acids*

Acid	COOH groups per molecule		Total number of acid groups per molecule	
	Calculated	Found by the Ca (oAc) ₂	Calculated	Found by the Ba(OH) ₂
o-phthalic	2.0	2.0	2.0	2.0
1,2,5-benzene-tricarboxylic	3.0	3.0	3.0	3.0
1,2,4,5-benzene-tetracarboxylic	4.0	4.0	4.0	4.0
Salicylic	1.0	1.0	2.0	2.0
2,4-dihydroxybenzoic	1.0	1.9	3.0	1.6
Gallic	1.0	2.5	4.0	3.8
2,5-dihydroxybenzoic	1.0	1.3	3.0	1.9

TABLE III: *Representative mixtures of AHA with Fe(III)^(a)*

	Initial iron and carbon percentage in reaction mixture ^(b)		Iron and carbon analysis of the product	
	Fe	C	Fe	C
II ^(c)	15.8	45.5	14.8	47.7
III	23.7	34.1	34.1	37.0
IV	28.5	27.3	27.3	30.6
V	31.7	22.7	29.2	24.2

^(a) See Fig. 4^(b) From the amounts added initially^(c) I corresponds to the blankTABLE IV: *Representative mixtures of AHA with Cu(II)^(a)*

	Initial copper and carbon percentage in reaction mixture ^(b)		Copper and carbon analysis of the product	
	Cu	C	Cu	C
II ^(c)	14.6	29.2	11.9	30.2
III	17.1	22.6	16.3	21.8
IV	20.4	13.5	21.2	11.6

^(a) See Fig. 5^(b) from the amounts added initially^(c) I corresponds to the blank

TABLE V: Comparison of free radical content of artificial and natural humic acids

Sample	10^{-18} spins g^{-1}
A. Artificial humic acid	10
B. Podzolic, B soil horizon, California Medecino Country soil (2% humic acid)	0.02
Part extracted into ethanol-benzene	1.0
water soluble fraction	0.8
alcohol soluble fraction	15.0
Fraction soluble in ethanol-water mixture	2.2
C. Podzolic, B soil horizon, England (Delaware Forest, Chesire)	
humic acid	0.2
humic acid hydrolyzed for 3h	0.5
humic acid hydrolyzed for 4h	0.9
D. Commercial sample (Fluka, Buchs, S.G.)	
humic acid	0.7
humic acid hydrolyzed for 3h	0.5

Περίληψη

Μελέτη της Χημείας των Χουμικών Όξέων. II. Άλληλεπιδράσεις Fe(III) και Cu(II) με πολυμερείς υποκαταστάτες που περιέχουν δευγόνο.

Η έρευνα της άλληλεπιδράσεως του Fe(III) και Cu(II) με την τολουοκινόνη και άλλα oligομερή προϊόντα της οξειδωτικής συμπεκνώσεως της παρουσία οξέων,¹ επεκτείνεται σε αυτήν εδώ την εργασία και σε πολυπλοκότερα πολυμερή μόρια, που μοιάζουν περισσότερο με φυσικά χουμικά συστατικά των εδαφών ή των υδάτων. Η ομοιότητα αυτή που αποδίδεται σε συγκεκριμένες κοινές δομές, αντανακλάται στα ηλεκτρονικά φάσματα (σχ. 2) καθώς και στα φάσματα υπεριώθρου (σχ. 3) και ESR (Πίν. V, σχ. 7), αλλά και σε ώρισμένες ιδιότητες που έχουν σχέση με τον ρόλο που παίζουν τα χουμικά οξέα στη φύση, όπως π.χ. η οξύτητά τους και οι χαρακτηριστικές τους ομάδες (Πίν. I, II, σχ. 1), ή ικανότητά τους να σχηματίζουν σύμπλοκα, κλπ.

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Studies on the Chemistry of Humic Acids III. Preliminary Observations on the Reduction of Dinitrogen by V(II) in the Presence of Humic Acids.

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Summary

Preliminary experiments show that in the presence of artificial or natural humic acids, V(II) in alkaline solutions reduces dinitrogen to ammonia. The existence in the humic acid of active groups in relative positions allowing chelate complex formation is a necessary prerequisite for the reduction. Provided that this condition is fulfilled, nitrogen fixation by mixtures containing V(II) and humic acids should to a large extent be independent of the composition and origin of these acids. The preliminary results reported here are compared to results from other systems which have been reported in the literature and are interpreted on the basis of a tentative generalized mechanism.

Key words: Humic acids, chelate complexes, dinitrogen fixation — reduction.

Introduction

The numerous studies on nitrogen fixation have been reviewed by several authors.¹⁻⁵ One of the objectives of these studies is the simulation in the laboratory of the action of nitrogenase by non-enzymatic model systems. Nitrogenase has been isolated⁶ from various soil microorganisms, both symbiotic and free-living. Underlying this effort is the assumption that biological nitrogen fixation via nitrogenase is the only path available under mild natural conditions. Here we explore the possibility for non-biological nitrogen fixation in the soil by a path involving humic acid without direct participation of microorganisms. The necessary background information has been reported in the first two papers⁷⁻⁸ of this series, where we studied the interaction between some metal ions and toluoquinone as well as the successive products of its oxidative 2,5 or 2,6 condensation to an artificial humic acid and made a comparison with natural humic acids.

The selection of the appropriate reducing metal ion was based on the work of Nikonova^{9,10} who demonstrated that vanadium (II) complexes with monomeric aromatic ligands having hydroxyl groups in neighboring positions (e.g. catechol, pyrogallol, gallic acid, 2,3-dihydroxynaphthalene, chromotropic acid, etc.) can reduce nitrogen.

Our artificial humic acid is not monomeric, and instead of having two or more neighboring hydroxyl groups, it has in neighboring positions one hydroxyl and one carbonyl group.

Natural humic acids also contain carboxyl groups. Yet, in spite of the differences, we find that both artificial and natural humic acids can play a role analogous to that of the ligands investigated by Nikonova.

Experimental

Materials: The preparation and/or purification of toluoquinone, toluohydroquinone and the dimer, trimer, polymer, etc. of toluoquinone, have been described.^{7,8} Here, in addition to these ligands we also tried several other commercially available hydroquinones, namely hydroquinone (p-dihydroxybenzene), resorcinol (m-dihydroxybenzene), catechol (o-dihydroxybenzene), 5-methylresorcinol (1,3-trihydroxybenzene). We also used protocatechuic acid (3,4-dihydroxybenzoic acid) and the polymers obtained from o- and p-benzoquinones.

Anhydrous $VCl_2 \cdot 2CH_3OH$ was prepared from $VCl_3 \cdot 6H_2O$. Ten grams of $VCl_3 \cdot 6H_2O$ were dissolved in 500 ml of ethyl or methyl alcohol and acidified slightly by passing gaseous HCl. 70 ml of pure benzene were then added and the mixture distilled. The remaining VCl_3 contained 0.08% water. 200 ml of a saturated solution of VCl_3 in freshly distilled alcohol (containing traces of iodine) were acidified with dry HCl (g) up to 10% in HCl and electrolyzed (12-15V, 1A, 25-30 h) in a deaerated cell having a porous container to prevent mixing of the chlorine produced with the V(II) solution. A platinum plate acted as a cathode and a carbon rod as anode. The inert atmosphere was sustained by purging the cell with oxygen-free, dry argon. Solid $VCl_2 \cdot 2CH_3OH$ can be obtained by removing the alcohol by distillation under high vacuum, but in most experiments the solution was used as such.

Solutions of VCl_2 in water-alcohol mixture were obtained by a similar procedure, except that in this case one can use concentrated instead of gaseous HCl.

Some solutions of V(II) in hydrochloric acid media were also obtained by reducing the corresponding V(III) solution with magnesium metal or zinc amalgam. The organic reagent was most of the times used in alkaline solution. After mixing with the acidic vanadous solution care was taken to have pH values in the range from 8 to 11. Fine adjustments were made with dilute aqueous or alcoholic hydrochloric acid or sodium hydroxide solutions.

Natural humic acid (NHA): We used two samples of natural humic acid obtained from the Soil Research Institute, Canada Department of Agriculture, Ottawa. We also separated two similar samples from Greek soil by a modification of the method developed by Schnitzer and Gupta.¹² Two kg of a forest soil sample were treated with ca. 7.1. of 1.5N NaOH at 50°C. The mixture was vigorously shaken for 8 hours in an argon atmosphere to avoid possible oxidation by air. The solution was then filtered and the filtrate, after being condensed, was passed through an ion exchange resin to remove cations and then evaporated to dryness at 50°C. The yield is small: (2 g from 2 kg of soil. Analysis: C 51.80, N 3.60, H 4.65. The sample used was retreated with few ml of warm NaOH 1N in order to be sure for the absence of any NH_3 traces.

General experimental procedure and analysis

A stream of nitrogen (1 atm) was passed through a series of purification traps, then through the reaction vessel and finally through another series of traps for analysis of the products. Only glass or polyethylene tubing was used for the connections. Nitrogen was purified by successively passing it through traps containing pyrogallol (in alkaline solution), $V^{2+}_{(aq)}$, a Katalyzator R-3-11 (purchased from B.A.S.F.), anhydrous $CaCl_2$, and silica gel. The reaction vessel was fitted with a syringe to admit the V(II) solution and with a thermometer. A

thermostat was used to control the temperature. The products were analyzed by passing through two traps filled with dilute hydrochloric acid (pH — 3) also containing an indicator. The first of these traps was also connected to a PH meter. Usually, any ammonia produced remained in the first trap, but in a few cases traces were also detected in the second trap.

Formation of ammonia is indicated by the change in pH and the color of the indicator in these two traps. It was further confirmed by adding alkali to the solution of the traps and then mildly heating. Gaseous ammonia was detected with an acid-base indicator, or by the Nessler reagent. In some cases it was also collected and titrated. Detection of hydrazine was done with p-dimethylaminobenzaldehyde.

Results and Discussion

The results of the experiments performed are summarized in Table I. Each run in this Table represents several experiments in duplicate or triplicate, under different conditions of pH, temperature etc. The time of purging nitrogen through the reaction mixture was typically 24 hours. All experiments were also successfully performed using ethanol instead of methanol.

Although the experiments were of a semi-quantitative nature it can still be stated that the yields, under otherwise identical conditions, were generally higher at higher temperatures and higher in methanol rather than in mixed methanol-water solvents.

Hydrazine was detected only in the first stages of run No. 4, but under the conditions of our experiments it is further reduced to ammonia.

Mixtures of V(II) with toluoquinone, toluohydroquinone, hydroquinone, o-benzoquinone, or resorcinol and its methyl derivatives gave no indication of nitrogen fixation. The corresponding experiments were performed in methanol, at a pH range between 9.5 and 10.5 and a temperature range between 25 and 30°C. In these experiments vanadous ion was prepared electrolytically. These negative results can be taken as blanks for the experiments in which nitrogen was reduced. Nevertheless, several other blanks were also performed in which nitrogen was passed through mixtures containing only the organic component or only V(II), or the organic components mixed with V(III) instead of V(II). Only in the blanks of vanadous prepared by reduction with magnesium metal were ammonia and hydrazine detected. In all other cases, including vanadous solutions (without the organic component) prepared electrolytically or by reduction of V(III) with Zn-Hg, results were negative.

Alkaline vanadous solutions prepared by reduction with magnesium contain $Mg(OH)_2$, which is coprecipitated with $V(OH)_2$ and it is known¹³ that vanadous in magnesium hydroxide gels can reduce nitrogen. With electrolytically prepared V(II) the reaction mixtures shown in Table I are homogeneous even at the higher pH used. The behavior of V(II) which was obtained by reduction with zinc amalgam is similar to that of V(II) obtained electrolytically, except that there is coprecipitation of zinc and vanadous hydroxides containing small amounts of the organic compound.

Under the conditions of our experiments, therefore, in the presence of monomeric compounds having hydroxyl or quinone groups in meta- or para-positions to each other, V(II) does not reduce dinitrogen. In contrast, this reduction takes place if the monomeric compounds have oxygen donor groups ortho- to each other. It is particularly interesting that polymeric products, even those obtained from inactive para-monomers, are active. Condensation obviously

TABLE I: Reaction mixtures yielding reduction^(a) of nitrogen to ammonia

Run	Mixture	pH range	Temperature range (C°)	Solvent
1	V(II), catechol ^(b)	9.5-10.5	25 - 60	methanol, methanol-water (2:1)
2	V(II), pyrogallol ^(b)	9.5-10.5	25 - 35	methanol, methanol-water (2:1)
3	V(II), protochatechuic acid ^(c)	9.5-10.5	25 - 55	methanol
4	V(II), artificial humic acid from toluoquinone	9.5-11	25 - 60	methanol, methanol-water (2:1)
5	V(II), artificial humic acid from o-benzoquinone	9.5-10.5	25 - 60	methanol-water (2:1)
6	V(II), natural humic acid	9.5-10.5	25 - 60	methanol-water (1:2, 1:1, 2:1)

(a) Mixtures of V(II) with toluoquinone, toluohydroquinone, hydroquinone, o-benzoquinone, resorcinol and its methyl derivatives gave no indication for nitrogen fixation.

(b) Confirming Shilov *et al.* loc. cit.

(c) Accompanied by decarboxylation

increases complex formation capacity by bringing active groups in relative positions allowing chelation and the evidence strongly suggests that this is the common characteristic distinguishing active from non-active compounds. The results further show that hydroxyl groups can partly be replaced in this capacity by quinone or carboxylic groups.

The significance of this simple conclusion is obvious. Most of the components in the complicated mixtures described by the collective name "natural humic substances" have neighboring active groups of this sort. It would then seem that nitrogen fixation under the appropriate conditions is quite a general property of these mixtures, to a large extent independent of composition and origin.

The mechanism of the non-enzymatic reduction of dinitrogen by V(OH)₂, which is known to be a powerful reducing agent, has been discussed by Denisov *et al.*,¹⁴ Lorenz *et al.*,¹⁵ and by Zones *et al.*,¹⁶ basically within the context of their experiments with V(OH)₂-Mg(OH)₂ gels. On the basis of their systematic investigation Zones *et al.* convincingly conclude that the reaction proceeds through a vanadium-nitride-type intermediate formed by a two-electron oxidative addition. This intermediate is subsequently hydrolyzed to cis-diimide, N₂H₂ and transformed further to hydrazine. Diimide is formed even in the absence of Mg(OH)₂, but in that case it decomposes into the elements, the decomposition being catalyzed by base,¹⁷ as well as by V(II) and V(III).

The basic premises of this mechanism and particularly the intermediate formation of diimide could also be used for explaining the results reported here. These results, however, clearly indicate that complexation — in fact chelation — is an important factor in stabilizing the intermediate reduction products and/or in reducing them further eventually all the way to ammonia. Mg(OH)₂ is therefore by no means unique in promoting nitrogen reduction. It is only a special case within the more general context explored here and elsewhere,^{9,10} which includes heterogeneous as well as homogeneous systems, hydroxyl, carbonyl and/or carboxyl complexing agents, solid host lattices, monomeric and polymeric ligands. The limited specificity of Mg(OH)₂ among the hydroxides so far investigated is probably due to its ability to provide an immediate environment around V(II) resembling the environment provided in the more general case by the active complexing agents.

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Περίληψη

Μελέτη τής χημείας τών χουμικών οξέων III. Προκαταρκτικές παρατηρήσεις επί τής αναγωγής του διαζώτου από V(II), παρουσία χουμικών οξέων.

Προκαταρκτικά πειράματα δείχνουν ότι παρουσία τεχνητών ή φυσικών χουμικών οξέων το V(II) σε αλκαλικά διαλύματα ανάγει το διάζωτο σε άμμωνία. Ύπαρξη ένεργών ομάδων σε κατάλληλες θέσεις, που να έπιτρέπουν σχηματισμό χηλικών συμπλόκων με το V(II), είναι απαραίτητη προϋπόθεση για την αναγωγή. Έφ' όσον ή προϋπόθεση αυτή εκπληροϋται, ή δέσμευση του άζώτου από μίγματα που περιέχουν V(II) και χουμικά οξέα πρέπει σε μεγάλο βαθμό να είναι ανεξάρτητη από τή σύνθεση και τήν προέλευση τών οξέων αυτών.

Τά προκαταρκτικά αποτελέσματα που περιγράφονται εδώ συγκρίνονται με τα αποτελέσματα από άλλα συστήματα που αναφέρονται στη βιβλιογραφία και έρμηνεύονται με βάση έναν πιθανό γενικό μηχανισμό.

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PREPARATION OF ISOXAZOLES FROM NITRILE OXIDES AND TRIMETHYLSILYL ENOL ETHERS VIA THE CORRESPONDING 5-TRIMETHYLSILYLOXY- Δ^2 -ISOXAZOLINES.

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Summary

The reactions of benzonitrile oxide and 2,6-dichloro-benzonitrile oxide with some trimethylsilyl enol ethers gave through a regioselective 1,3-dipolar cyclo addition 5-trimethylsilyloxy- Δ^2 -isoxazolines which were converted to isoxazoles by treatment with hydrochloric acid. Silyl enol ethers prepared from aldehydes gave 4-substituted isoxazoles while those prepared from ketones gave 5-mono- or 4,5-bis-substituted isoxazoles. The structure of the new isoxazoles is confirmed by their spectral data ($^1\text{H-NMR}$, MS).

Key words: 5-Trimethylsilyloxy- Δ^2 -isoxazolines, regioselectivity, 1,3-dipolar cycloaddition, nitrile oxides, substituted isoxazoles preparation-spectroscopy, silyl enol ethers.

Introduction

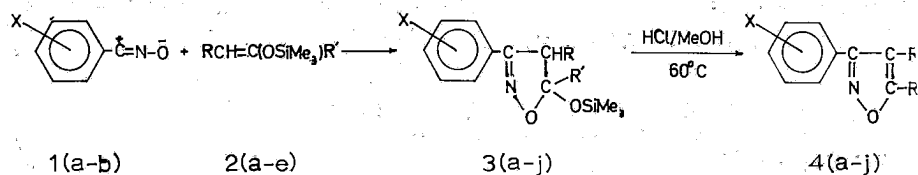
The isoxazole ring is mainly synthesized through the long known methods of condensing 1,3-dicarbonyl compounds, α -acetylenic ketones or aldehydes with hydroxylamine and also directly through the reaction of nitrile oxides with acetylenes.^{1,2} Isoxazoles are also synthesized via the appropriate, more or less stable, Δ^2 -isoxazolines prepared through the reaction of nitrile oxides with enamines,^{3,4} enol derivatives⁵ and other olefins substituted in the α -position with a suitable leaving group.⁶ Δ^2 -isoxazolines can also be converted to isoxazoles by oxidation.⁷

Recently⁸ we have studied reactions between the nitrile oxides *1(a,b)* and the trimethylsilyl enol ethers $\text{CH}_3\text{COCH}=\text{C}(\text{OSiMe}_3)\text{CH}_3$, $\text{C}_6\text{H}_5\text{COCH}=\text{C}(\text{OSiMe}_3)\text{CH}_3$, *2a, 2e* and found that in all cases a regioselective 1,3-dipolar cycloaddition of the nitrile oxide to the dipolarophile occurs, leading originally to the formation of the corresponding 5-trimethylsilyloxy- Δ^2 -isoxazolines *3*. The isoxazolines *3* (with $\text{R} = \text{CH}_3\text{CO-}$ or $\text{C}_6\text{H}_5\text{CO-}$ and $\text{R} = \text{CH}_3$), prepared from the above first two dipolarophiles, were generally unstable and converted to the corresponding isoxazoles *4*, before their isolation, while isoxazolines *3(a,i,j)* (Table I), prepared from *2a, 2e*, were stable.

It is now reported that these stable isoxazolines can be easily converted to the corresponding isoxazoles by short treatment with methanolic hydrochloric acid and also that the reaction between nitrile oxides and trimethylsilyl enol ethers can be finally utilized for the preparation of isoxazoles, alkyl- or aryl-substituted in the 4- or 5- or both positions of the isoxazole ring, depending on the trimethylsilyl enol ether used.

Results and discussion

In this paper we wish to report a convenient synthesis of isoxazoles 4(a-j) via the corresponding 5-trimethylsilyloxy- Δ^2 -isoxazolines 3(a-j) (Table I), according to the following general reaction scheme:



1a: X = H

b: X = 2,6-Di-Cl

2a: R = H, R' = C₆H₅

b: R = R' = CH₃ (cis:trans 30:70)

c: R = H, R' = C₂H₅

d: R = C₂H₅, R' = H (cis:trans 60:40)

e: R-R' = -(CH₂)₄- (>98%)⁹

Silyl enol ethers are easily prepared in good yields by several methods through the silylation of carbonyl compounds.¹⁰ Trimethylsilyl enol ethers 2(a-e) were prepared by treatment of the corresponding ketone or aldehyde with triethylamine and chlorotrimethylsilane⁹ and were used in the reactions studied in the form of isomeric mixtures. In all reactions the nitrile oxide 1 was added in excess (two equivalents). In method A the benzonitrile oxide 1a was prepared *in situ*, while in method B freshly prepared 2,6-dichloro-benzonitrile oxide 1b was used. Method B gave generally better yields (Table I). Compounds 3 were isolated from the reaction mixture by column chromatography in a crude form, usually as mixtures with some of the corresponding furoxan (dimerization product of 1) and were converted to the corresponding isoxazoles 4 directly, during the separation procedure or upon short heating with 10% HCl in methanolic solution. It is interesting to note that the reaction of the *cis:trans* isomeric mixture of the silyl enol ether 2d with the nitrile oxide 1a resulted probably to the formation of only one isomer for the corresponding Δ^2 -isoxazoline 3g and not to a mixture of the two possible stereoisomers, as it is indicated by the ¹H-NMR spectrum of the cycloaddition product. The same was also observed in the reaction between 2d and 1b. The ¹H-NMR spectra (in CCl₄) of products 3g and 3h showed for their C₅-H proton only one doublet at $\delta = 5.97$ (J = 6.5 Hz) and 5.87 ppm (J = 6 Hz) respectively.

The prepared known isoxazoles, with exception of isoxazole 4b (see Tables I, II), are identical to those reported in the literature, while the structure of the new compounds 4 is confirmed by their spectral data (¹H-NMR, MS). Compounds 4(a,b,d,f) show in the ¹H-NMR spectra a peak at $\delta = 6.0 - 6.5$ ppm (Table II), characteristic for their C₄-H proton,^{11,12,13} compounds 4(g,h) show a peak at $\delta = 8.20$ ppm, characteristic for their C₅-H proton^{12,13} and compounds 4(c,e) show two equal singlets at $\delta = 2.4$ and 2.0 ppm for the protons of their two methyl groups. Although the ¹H-NMR spectrum of compound 4b differs significantly to that reported in the literature¹⁴ (Table II), we consider that the observed chemical shift for its C₄-H proton at $\delta = 6.53$ ppm is in good agreement with the proposed structure, since the same proton of compound 4a resonates at $\delta = 6.73$ ppm. Table II shows that the same difference is also observed in chemical shifts of the C₄-H proton of isoxazoles 4f ($\delta = 5.98$ ppm) and 4d ($\delta = 6.22$ ppm).

TABLE I. Isoxazoles 4 prepared from 1 and 2 via the corresponding 5-Trimethylsilyloxy- Δ^2 -isoxazolines 3.

Preparation of 3			X	R	R'	Compounds 4		Molecular formula	Calculated/Found			
Starting Materials		Method				Yield ^a (%)	m.p.		Recryst. Solvent	Lit.m.p. (b.p.)	%C	%H
1	2	Reaction time (h)										
a	1a	2a	A (12)	H	H	C ₆ H ₅	45	142-143° CCl ₄	C ₁₅ H ₁₁ NO 141 ^{6,17}			
b	1b	2a	B (30)	2,6-Di-Cl	H	C ₆ H ₅	36	58-59° CCl ₄ /P. Ether	C ₁₅ H ₉ Cl ₂ NO 75-76 ^{6,14}	62.09 61.95	3.13 3.23	4.83 5.03
c	1a	2b ^b	A (10)	H	CH ₃	CH ₃	5	oil (GLC) ^c	C ₁₁ H ₁₁ NO ^d	76.27 76.68	6.40 6.52	8.09 7.51
d	1a	2c ^b	A (10)	H	H	C ₂ H ₅	35	oil (GLC) ^c	C ₁₁ H ₁₁ NO ^d oil (GLC) ¹⁸			
e	1b	2b ^b	B (48)	2,6-Di-Cl	CH ₃	CH ₃	32	95-96° CH ₃ OH	C ₁₁ H ₉ Cl ₂ NO ^e	54.57 54.56	3.75 3.73	5.78 5.76
f	1b	2c ^b	B (48)	2,6-Di-Cl	H	C ₂ H ₅	46	oil (GLC) ^c	C ₁₁ H ₉ Cl ₂ NO ^e	54.57 54.34	3.75 3.92	5.78 5.64
g	1a	2d	A (10)	H	C ₂ H ₅	H	4	oil (GLC) ^c	C ₁₁ H ₁₁ NO (155°/13 torr) ¹³			
h	1b	2d	B (36)	2,6-Di-Cl	C ₂ H ₅	H	12	oil (GLC) ^c	C ₁₁ H ₉ Cl ₂ NO	54.57 54.89	3.75 3.85	5.78 5.39
i	1a	2e	A (12)	H		-(CH ₂) ₄ -	7	52-53° C ₂ H ₅ OH/H ₂ O	C ₁₃ H ₁₃ NO 53-54 ^{6,3}			
j	1b	2e	B (30)	2,6-Di-Cl		-(CH ₂) ₄ -	17	123-124° (C ₂ H ₅) ₂ O	C ₁₃ H ₁₁ Cl ₂ NO	58.23 58.25	4.13 4.09	5.22 5.23

a: Total yield of 4 based on dipolarophile 2 used.

b: A mixture of 2b:2c in the 80:20 ratio was used.

c: See experimental, preparation of compounds 4.

d: Isoxazoles 4c and 4d were prepared in mixture and separated by column chromatography. Isoxazole 4d was eluted first.

e: Isoxazoles 4e and 4f were prepared in mixture and separated by column chromatography. Isoxazole 4f was eluted first.

Compounds 4(a-j) gave in their mass spectra the expected molecular ion () with a high relative intensity and in some cases as the base peak (Table II). The general fragmentation process of the compounds studied resembles that reported for similar isoxazoles,^{15,16} involving in the case of the 5-substituted isoxazoles 4(a,b,d,f) and the 4,5-dimethyl-isoxazoles 4(c,e) mainly the loss of the substituent R' (M - R' peak), the loss of the radical R'C=O (M - R'CO peak) and the formation of the cation R'-C=O⁺ and in the case of the 4-substituted isoxazoles 4(g,h) the loss of R (M - R peak), RC=C=O (M - RC=C=O peak) and CO (or C₂H₄) (M - 28 peak). Loss of stable neutral fragments (C₂H₄) was observed in compounds 4(i,j) where the 4- and 5- positions of the isoxazole ring are bridged by an aliphatic side chain.

From the reactions studied it is evident that the pronounced directional effect of the trimethylsilyloxy group in the 1,3-dipolar cycloaddition of nitrile oxides to trimethylsilyl enol ethers can be utilized for the preparation of suitable substituted isoxazoles, via the corresponding 5-trimethylsilyloxy- Δ^2 -isoxazolines. Silyl enol ethers prepared from aldehydes lead in this way to the formation of 4-substituted isoxazoles, while those prepared from ketones lead to the formation of 5-mono- or 4,5-bis-substituted isoxazoles.

TABLE II. NMR and MS spectral data of Compounds 4(a-j).

Compound	¹ H-NMR (CCl ₄) δ ppm	MS m/e (relative intensity, ion)
4a	7.95-7.32 (m, 10H); 6.73 (s, 1H, C ₄ -H).	221 (63,), 193 (6), 144 (16, M - C ₆ H ₅), 116 (4, M - C ₆ H ₅ CO), 105 (100, C ₆ H ₅ CO), 77 (54, C ₆ H ₅), 51 (16).
4b	7.97-7.30 (M, 8H); 6.53 (s, 1H, C ₄ -H). ^a	289 (32,), 254 (11, M - Cl), 212 (11, M - C ₆ H ₅), 184 (4, M - C ₆ H ₅ CO), 105 (100, C ₆ H ₅ CO), 77 (45), 51 (12).
4c	7.75-7.30 (m, 5H); 2.37 (s, 3H, C ₅ -CH ₃); 2.06 (s, 3H, C ₄ -CH ₃).	173 (100,), 158 (45, M - CH ₃), 131 (24, M - CH ₂ CO) 130 (36, M - CH ₃ CO), 77 (26), 51 (14), 43 (14, CH ₃ CO)
4d	7.88-7.32 (m, 5H); 6.22 (s, 1H, C ₄ -H), 2.80 (q, 2H, J=7.5 Hz). 1.36 (t, 3H, J=7.5 Hz).	173 (87,), 145 (31), 144 (100, M - C ₂ H ₅), 117 (15) 116 (39, M - C ₂ H ₅ CO), 77 (58), 57 (12, C ₂ H ₅ CO), 51 (28).
4e	7.37 (br.s, 3H); 2.42 (s, 3H, C ₅ -CH ₃); 1.78 (s, 3H, C ₄ -CH ₃).	241 (100,), 226 (91, M - CH ₃), 199 (64 M - CH ₂ CO) 198 (53, M - CH ₃ CO), 174 (17), 173 (22), 172 (27), 171 (28), 43 (49, CH ₃ CO).
4f	7.34 (br.s, 3H); 5.98 (s, 1H, C ₄ -H); 2.87 (q, 2H, J=7.5 Hz), 1.38 (t, 3H, J=7.5 Hz).	241 (31,), 226 (7, M - CH ₃), 212 (100, M - C ₂ H ₅), 184 (22, M - C ₂ H ₅ CO), 173 (14), 57 (8, C ₂ H ₅ CO).
4g	8.18 (br.s, 1H, C ₅ -H); 7.77-7.32 (m, 5H); 2.60 (q, 2H, J=7.5 Hz), 1.23 (t, 3H, J=7.5 Hz).	173 (59,), 158 (99, M - CH ₃), 146 (16), 145 (15, M - 28), 144 (36, M - C ₂ H ₅), 130 (62), 118 (71), 104 (21, M - C ₂ H ₅ C=CO), 103 (28), 77 (100).
4h	8.29 (br.s, 1H, C ₅ -H); 7.40 (br.s, 3H); 2.30 (q, 2H, J=7.5 Hz); 1.13 (t, 3H, J=7.5 Hz).	241 (60,), 226 (100, M - CH ₃), 214 (9), 213 (13, M - 28), 212 (13, M - C ₂ H ₅), 206 (15, M - Cl), 198 (49), 178 (67), 173 (12), 172 (20, M - C ₂ H ₅ C=CO), 171 (13).
4i	7.83-7.30 (m, 5H); 2.87-2.48 (m, 4H); 2.03-1.69 (m, 4H).	199 (100,), 198 (60), 171 (18), 170 (44); 143 (75), 130 (20), 129 (23), 119 (46), 117 (15), 103 (35), 102 (21), 101 (22), 77 (68), 75 (40), 51 (32).
4j	7.37 (br.s, 3H); 2.92-2.63 (m, 2H); 2.45-1.67 (m, 6H).	267 (100,), 239 (9), 238 (7), 232 (36, M - Cl), 213 (29), 211 (45), 204 (64), 198 (25), 174 (18), 173 (13), 172 (26).

a: Lit.¹⁴ δ = 7.35-6.65 (m, ArH), 6.10 ppm (s, 1H, C₄-H) (in CDCl₃)

Experimental Part

M.p.'s. are given without correction and were determined with a Kofler hot-stage apparatus. $^1\text{H-NMR}$ spectra were obtained in carbon tetrachloride with a Varian A-60A spectrometer with tetramethylsilane as internal standard. The mass spectra were obtained with a Hitachi Perkin-Elmer RMU-6L mass spectrometer; the ionization energy was maintained at 70 eV. Earlier reported procedures were used for the preparation of the benzhydroximic acid chloride,¹⁹ 2,6-dichlorobenzonitrile oxide²⁰ and trimethylsilyl enol ethers 2(a-e).⁹ Compounds 2a, 2d, 2e were prepared by silylation of acetophenone, butyraldehyde and cyclohexanone respectively, while compounds 2b and 2c were prepared as a mixture in the ratio 80:20 by the silylation of butanone. Compounds 2b and 2d were prepared and used in the form of *cis:trans* isomeric mixtures given in the reaction scheme.

General Procedure for the Preparation of 5-Trimethylsilyloxy- Δ^2 -isoxazolines 3(a-j).

Method A: In this method the benzonitrile oxide 1a was prepared *in situ* from benzhydroximic acid chloride. To a stirred and ice-cooled ethereal solution (70 ml) of benzhydroximic acid chloride (18 mmol) and dipolarophile 2 (9 mmol) a stoichiometric amount (18 mmol) of triethylamine in ether (30 ml) was added dropwise during 1 h. The reaction mixture was stirred for further 2 h under cooling and then for 10 h at room temperature. The triethylamine hydrochloride was filtered off, the filtrate was partly concentrated under reduced pressure and petroleum ether was added to precipitate most of the produced furoxan. The furoxan was filtered off, the solvent was evaporated and the residue was chromatographed over silica gel, using hexane / ether gradient elution.

Method B: An ethereal solution (50 ml) of freshly prepared 2,6-dichloro-benzonitrile oxide 1b (18 mmol) and the dipolarophile 2 (9 mmol) was heated under reflux (30 - 48 h). The reaction was monitored by T.L.C. The solution was partly concentrated, petroleum ether was added to precipitate most of the produced furoxan and the mixture was then worked-up as in method A.

In both methods some furoxan was usually eluted very close to, or along with the cycloaddition product 3. The content of each fraction was checked by $^1\text{H-NMR}$. Generally compounds 3 were more or less slowly converted to the corresponding isoxazoles 4 during the separation and purification procedures as well as during prolonged standing.

General Procedure for the Preparation of Isoxazoles 4(a-j).

In a methanolic solution of the above isolated crude compounds 3 10% hydrochloric acid was added and the mixture was heated for 10 min at 60° C. The cooled reaction mixture was diluted with water and extracted with chloroform. The water-washed and dried (Na_2SO_4) extract was evaporated and the residue was chromatographed on silica gel using light petroleum ether/ether (85:15) mixtures as eluent; each fraction was checked by $^1\text{H-NMR}$. The liquid products 4c, 4d, 4f and 4h, listed in the Table I, were further purified by G.L.C. on a Silicon SE 30, 2 $\frac{1}{4}$ ft column at 162°, 177°, 177° and 175° C respectively, while the liquid 4g was also purified by G.L.C. on a similar 4 ft column at 155° C.

Περίληψη

Παρασκευή Ισοξαζολίων από Νιτριλοξειδία και Τριμεθυλοσιλυλο-ενολαιθέρες, δια μέσου των αντίστοιχων 5-Τριμεθυλοσιλυλοξυ- Δ^2 -ισοξαζολινών.

Στήν εργασία αυτή περιγράφεται ή σύνθεση των ισοξαζολίων 4(a-j) δια μέσου των 5-τριμεθυλοσιλυλοξυ- Δ^2 -ισοξαζολινών 3(a-j). Οί ενώσεις 3(a-j) παρασκευάζονται από τη στερεοεκλεκτική (regioselective) 1,3-διπτολική κυκλοπροσθήκη των νιτριλοξειδίων 1(a,b) στους τριμεθυλοσιλυλο-ενολαιθέρες 2(a-e) και μετατρέπονται στα αντίστοιχα ισοξαζόλια 4(a-j) με την επίδραση μεθανολικού διαλύματος ύδροχλωρικού όξέος. Όρισμένες από τις ενώσεις 3 είναι άρκετά άσταθείς και μετατρέπονται σε ενώσεις 4 άμέσως μετά τδ σχηματισμό τους. Η δομή που προτείνεται για τὰ νέα ισοξαζόλια συμφωνεί με τὰ φάσματα $^1\text{H-NMR}$ και τις χαρακτηριστικές διασπάσεις που παρατηρούνται στα φάσματα μάζης τους.

Σά συμπέρασμα προκύπτει ότι με τήν μέθοδο αυτή παρασκευάζονται εύκολα άλκυλο- και άρυλο-ύποκατεστημένα ισοξαζόλια. Οί σιλυλο-ενολαιθέρες που προέρχονται από άλδεϋδες δίνουν τελικά 4-ύποκατεστημένα ισοξαζόλια, ενώ οί σιλυλο-ενολαιθέρες που προέρχονται από κετόνες δίνουν 5-μόνο- ή 4,5-δι-ύποκατεστημένα ισοξαζόλια.

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A Method of Repeated Equilibrations to determine Maximum Retention Capacities for Ammonium and Equilibrium Coefficients in Soils

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Summary

By equilibrating repeatedly the same soil sample with fresh portions of a dilute ammonium salt solution, a wide range of ammonium concentrations in the soil phase can be achieved, with little variations in the total ionic concentration of the outer solution. Changes in the activity coefficients of the ions in the solution are thus minimized, and by considering the repeated equilibrations as a series of ion exchange equilibria an equation is derived which can be used to plot the experimental data in a linear form. The maximum amount of ammonium that can be retained by the soil, and the relevant ion exchange equilibrium coefficient can be determined from such a plot.

The method is applied to 4 soil Orders and it is shown that the experimental results conform to the equation derived. The exchange equilibrium coefficients and the maximum retention capacities of the various soil Orders studied were found to be characteristic of each Order, thus providing a new parameter for soil evaluation in relation to ammonium fertilizers application.

Key words, Repeated equilibrations in soils, Ammonium in soils, Ion exchange in soils.

INTRODUCTION

When a soil sample is in contact with an ionic solution ion migration occurs from one phase to the other, and this phenomenon has been the object of numerous investigations, since it has a great theoretical and practical importance, e.g. in fertilizing soils. In most of the cases studied the theoretical analysis of the results has been based either on the notion of adsorption at a solution-solid interphase, or on ion exchange equilibria between the two phases in contact. Adsorption at a solution-solid interphase is usually described by the Freundlich equation, but this has the disadvantage of not providing the means to calculate the adsorption maximum. This is why equations of the Langmuir type are usually employed, and these permit the calculation of the adsorption maximum. However, the assumptions underlying the Langmuir equation (localized monolayer on a uniform surface with no lateral interactions etc.) are rarely met in soils, and therefore the use of such an equation seems rather unrealistic, being only an empirical method to analyze the experimental data. Curved Langmuir plots have often been interpreted as adsorption on two types of sites.^{1,2,3}

Various equations describing ion exchange equilibria have been derived and these have been reviewed by Helfferich,⁴ while their applicability to soils discussed by Babcock.⁵ The main problem here seems to be the variation of the equilibrium coefficient (equilibrium quotient) with the total concentration of the solution and with the ratio of the amounts of the competing ions. This is usually attributed to the variation of the activity coefficients of the ions in the outer solution and in the solid. Thermodynamic equilibrium constants can be calculated as described by Gaines and Thomas.⁶

We were interested in the ability of Greek soils to adsorb ammonium from solution, and it occurred to us that by repeated equilibrations of the same soil sample with fresh portions of the same dilute ammonium salt solution, a wide range of ammonium concentrations in the soil phase can be achieved with little variations in the total ionic concentration of the outer solution. Under these conditions changes in the activity coefficients of the ions in the solution are minimized, and if the change in the ratio of the activity coefficients of the competing ions in the soil complex is small, the repeated equilibrations mentioned above can be used to determine both, the maximum amount of ammonium which can be retained by the soil and the relevant equilibrium coefficient. This can be done by considering the repeated equilibrations as a series of ion exchange equilibria, and deriving on this basis an equation to analyse the experimental data.

Theoretical

Consider the constituent RA_z of the soil, containing the exchangeable cation A with valence z , in contact with a solution of an ammonium salt. We assume that adsorption of solvent and of negative ions from solution by the soil is negligible, and that the following ion exchange equilibrium is established with each fresh portion of ammonium salt solution:



The equilibrium coefficient for the n th equilibration, expressed in terms of molar concentrations for the outer solution and equivalent fractions for the solid, can be written as

$$K = \frac{(x_n/z) (N_{\text{NH}_4})_n^z}{(c - x_n)^z (N_A)_n} \quad (2)$$

where:

- c = initial concentration of each portion of ammonium solution (mol. l^{-1});
- x_n = decrease in the initial concentration of the ammonium solution in the n th equilibration (mol. l^{-1});
- $(N_{\text{NH}_4})_n, (N_A)_n$ = equivalent fractions of the ions NH_4^+ and A^{z+} , respectively, in the solid after the n th equilibration (dimensionless).

The equivalent fraction $(N_{\text{NH}_4})_n$ can be set equal to the expression

$$(N_{\text{NH}_4})_n = \frac{(V/w) \sum_{i=1}^n x_i}{q} \quad (3)$$

where

- $\sum_{i=1}^n x_i$ = cumulative concentration of ammonium removed from the n portions of solution after n equilibrations (mol. l^{-1});
- V = volume of each portion of solution (l);
- w = weight of soil sample (kg);
- q = number of equivalents of exchange sites per kg of soil, which is equal to the maximum amount of ammonium that can be retained by the soil (mol NH_4^+ per kg soil).

By substituting in Eq. (2) $1-(N_{NH_4})_n$ for $(N_A)_n$, and the R.H.S. of Eq. (3) for $(N_{NH_4})_n$, one obtains after rearrangement

$$x_n \left[\frac{\sum_{i=1}^n x_i}{c - x_n} \right]^z = z K \left(q \frac{w}{V} \right)^z - z K \left(q \frac{w}{V} \right)^{z-1} \sum_{i=1}^n x_i \quad (4)$$

Thus, if K does not change appreciably from one equilibration to the other, we can choose a suitable value for z (1,2 or rarely 3) and plot the L.H.S. of Eq. (4) versus $\sum x_i$, obtaining a straight line. The slope and the intercept of this line will permit the computation of $q \left(\frac{w}{V} \right)$ and K by means of the relations:

$$q \left(\frac{w}{V} \right) = - \frac{\text{intercept}}{\text{slope}}, \quad \text{and } K = \frac{|\text{slope}|^z}{z (\text{intercept})^{z-1}} \quad (5)$$

The value of q is then calculated from the known ratio w/V (kg/l).

Experimental

Soil Samples

The soil samples were from selected Alfisols (Nos. 28, 29), Vertisols (Nos. 34, 39), one Entisol (No. 54) and one Inceptisol (No 60). They were obtained from different regions of the Country and from the first horizon of classified profiles. Their characteristic properties as well as the depth from which they were obtained, are given in Table I.

After air drying the samples were passed through a 1000 μ sieve. All determinations and adsorption experiments were carried out on this soil fraction. Determinations for soil characterization were done according to the established methods of soil analysis.^{7,8}

The easily exchangeable NH_4^+ was determined as follows. A soil sample (3 g) was brought in a 50 cm^3 PVC centrifuge tube with 30 cm^3 NH_4Cl 1N, pH 7.0, and agitated on a reciprocating shaker for 30 min. After centrifuging, the supernatant liquid was decanted off. This was repeated 3 times. The remaining soil precipitate was thoroughly washed once with ethanol and 6-8 times with acetone (till free of Cl^-). Then the soil was treated with KCl 2N, pH 7.0 in the same way as above. The supernatant phase after each centrifugation was transferred in a 100 cm^3 volumetric flask and made to volume with KCl . The exchanged NH_4^+ in this solution was determined by the Kjeldahl method.

Adsorption experiments

The soil sample (3 g) was repeatedly (15 times) equilibrated in a stoppered PVC centrifuge tube with a portion of 0.01 or 0.02 mol. l^{-1} solution of ammonium chloride, so that a soil: solution ratio of 1:5, 1:10 and 1:15 was obtained. The

TABLE I: *Some soil characteristics*

Soil sample	Soil Order	Depth (cm)	Texture	Horizon	Soil paste pH	CaCO ₃ (%)	Cation exch. capacity (me/kg)	Easily exch. NH ₄ ⁺ (me/kg)
28	alfisol	0-5	loam	—	7.1	0	100	71
29	alfisol	5-30	loam	—	6.5	0	60	45
34	vertisol	0-20	clay	Ap	7.0	0	328	286
39	vertisol	0-20	clay	Ap	7.4	0	396	390
54	entisol	0-24	loam	Ap	7.4	12.8	244	75
60	inceptisol	0-14	clay-loam	Apl	7.5	17.1	336	102

equilibration was attained by shaking the mixture on a reciprocating shaker for 10 min. It was found by experimentation that after 6 min shaking the equilibration was practically complete, and that increasing the time of shaking beyond this did not have any effect on the results. After that, the sample was centrifuged at 3000 rpm, the supernatant liquid phase was transferred to a PVC bottle with a drop of phenyl mercuric acetate preservative to avoid ammonium oxidation. Before ammonium determination the extracts of every equilibration series were kept in 5° C for 24 hr.

Ammonium was determined by the conventional steam distillation method in a special distillation apparatus.⁹

The distillate was received in a 2% solution of boric acid containing a mixture of bromocresol green and methyl red indicator, and the ammonium was titrated with 0.01 N sulfuric acid solution.

All experiments were run in duplicate. The variation of the values obtained were not statistically significant and were within the limits of experimental error. Their mean value was used in all calculations.

Results and Discussion

Examples of the use of Eq. (4) to plot experimental data are shown in Figure 1. These results were obtained with soil sample No 34 (a Vertisol) by using a different w/V ratio in each run, and with two ammonium solution concentrations. It is seen from the Figure that all results conform to Eq. (4) with $z = 2$. In Tables II and III we give the values of q and K , respectively, which were calculated from the slopes and intercepts of such plots by standard least-squares procedures. The errors given with each q or K value were found by the method of propagation of errors.

The results compiled in these two Tables show that the differences in the values of q or K found from experiments with different soil to solution ratios, and with ammonium solution concentrations differing by a factor of 2, are not statistically significant in most cases. This indicates that the assumptions made in the derivation of Eq. (4) are realized experimentally, in spite of the high heterogeneity of the soils. One of these assumptions, namely that adsorption of negative ions from solution is negligible, was confirmed experimentally by determining the concentration of chloride ion in the solution phase after each equilibration with fresh portion of ammonium chloride solution. No change in the chloride ion concentration was detected. Also, the soil was found to adsorb a negligible amount of solvent during the repeated equilibrations. Changes in the ratios of the activity coefficients of the competing ions in the solution and/or the solid phase may occur, but these obviously lie within the fiducial limits laid by the experimental errors and the various computations of q and K .

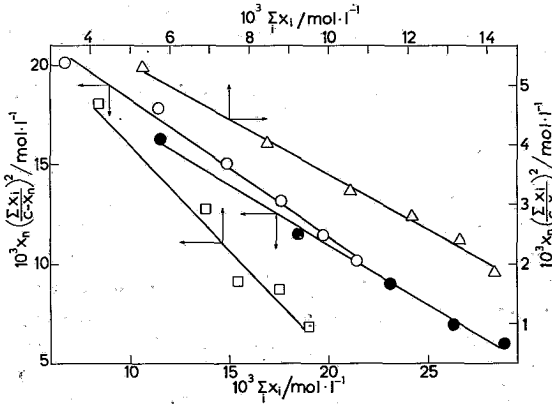


FIG. 1. The results of repeated equilibrations of the soil sample No 34 with two ammonium chloride concentrations (c), plotted according to Eq. (4).
 O, ●: $w/V = 1/5 \text{ kg.l}^{-1}$; □: $w/V = 1/10 \text{ kg.l}^{-1}$;
 □ (left ordinate divided by 10): $w/V = 1/15 \text{ kg.l}^{-1}$;
 empty symbols correspond to $c = 10.6 \text{ mmol.l}^{-1}$, while filled-in symbols to $c = 21.2 \text{ mmol.l}^{-1}$.

TABLE II: Maximum retention capacities for ammonium (q / mmol per kg soil) of various soils, determined by plotting experimental data, according to Eq. (4). Three ratios w/V (kg/l) and two ammonium chloride concentrations (c /mmol. l^{-1}) were used. The temperature of the experiments was 25°C . All errors associated with the q values are standard errors, calculated by regression analysis

Soil sample	z	$w/V = 1/5$		$w/V = 1/10$	$w/V = 1/15$
		$c = 10.6$	$c = 21.2$	$c = 10.6$	$c = 10.6$
28 (alfisol)	1	26 ± 2	32 ± 3	36 ± 1	37 ± 6
29 (alfisol)	1	22 ± 1	—	28 ± 3	25 ± 3
34 (vertisol)	2	182 ± 9	190 ± 10	195 ± 8	190 ± 20
39 (vertisol)	2	200 ± 10	—	231 ± 5	240 ± 15
54 (entisol)	1	—	75 ± 5	66 ± 5	—
60 (inceptisol)	1	—	98 ± 3	70 ± 10	—

TABLE III: Equilibrium coefficients (K) at 25°C , for the exchange adsorption of ammonium by soils from aqueous ammonium chloride solutions of concentration c /mmol. l^{-1} , and w/V soil to solution ratio (kg/l). The K values and their associated standard errors were calculated by regression analysis from plots of experimental data according to Eq. (4)

Soil sample	z	$w/V = 1/5$		$w/V = 1/10$	$w/V = 1/15$
		$c = 10.6$	$c = 21.2$	$c = 10.6$	$c = 10.6$
28 (alfisol)	1	0.22 ± 0.01	0.090 ± 0.007	0.15 ± 0.01	0.11 ± 0.01
29 (alfisol)	1	0.230 ± 0.008	—	0.14 ± 0.01	0.15 ± 0.01
34 (vertisol)	2	9.5 ± 0.9	7.9 ± 0.7	9.5 ± 0.7	8 ± 2
39 (vertisol)	2	15 ± 1	—	12.7 ± 0.5	9 ± 1
54 (entisol)	1	—	0.53 ± 0.02	0.39 ± 0.02	—
60 (inceptisol)	1	—	0.38 ± 0.01	0.51 ± 0.07	—

As it is seen in Table II the maximum retention capacities for ammonium, q , seem to be characteristic of each soil and are comparable to the C.E.C. and to the easily exchangeable ammonium (E.E.A.) of Table I. It is, however, noteworthy that q is found always smaller than these two parameters, and this probably reflects a basic difference of methodology for determining q and C.E.C. or E.E.A. For q we employed a certain number of equilibrations with a dilute ammonium solution, comparable in concentration with the ionic strength of the soil solution *in situ* ($ca 10^{-2}$), and calculated its value by a virtual extrapolation using mathematical relations. By contrast, the C.E.C. and E.E.A. were found by saturating the soil with solutions having 100 fold greater concentrations, and these undoubtedly influence the values of C.E.C. and E.E.A. found. One therefore is inclined to believe that the method of determining maximum retention capacities, as developed here, is more reliable, thus providing a measure of the fate of the fertilizer ammonium added in the soil. In fact, we see that the Vertisols act as a better reserve of the ammonium ion, preventing leaching, oxidation etc., thus permitting a better use of it by the plants.

The equilibrium coefficient, K , for the exchange of ammonium (Table III) gives a characteristic parameter of the affinity of the soil Order for binding ammonium. This parameter is independent of variations in soil to solution ratio and ionic concentration of the environmental solution. Moreover, the application of Eq. (4) for $z = 1$ or $z = 2$ gives information as to the kind of cations adsorbed in the soil complex of each soil Order. This is confirmed by determining the exchangeable cations of the various soils. For the cases with $z = 1$ the monovalent were found to outweigh the divalent cations, whilst when $z = 2$ the divalent were found in considerable excess. For example, for the Alfisol No 28, the monovalent (Na+K) were 5.07 and the divalent (Ca + Mg) 2.80 mmol/kg. For Vertisol No 34 the Na+K were 2.63 and the Ca + Mg 9.95 mmol/kg.

The fact that $z = 1$ for Entisol No 54 and Inceptisol No 60, in spite of the fact that these two soils contain free $CaCO_3$ (cf. Table I), seem to suggest that the monovalent cations of the soil complex do not exchange easily with Ca^{++} .

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Περίληψη

Η μέθοδος των επανειλημμένων έκχυλλίσεων για τον προσδιορισμό της μεγίστης ικανότητας συγκρατήσεως αμμωνίου από έδαφη, καθώς και των σχετικών συντελεστών ισορροπίας

Με επανειλημμένες εξισορροπήσεις του αυτού δείγματος εδάφους με νέα κάθε φορά ποσότητα αραιού διαλύματος ενός αμμωνιακού άλατος, επιτυγχάνεται μία περιογή συγκεντρώσεων προσροφούμενου αμμωνίου στην έδαφική φάση, με μικρές μόνο μεταβολές της ολικής ιονικής συγκεντρώσεως του εξωτερικού διαλύματος. Έτσι οι μεταβολές των τιμών των συντελεστών ενεργότητας των ιόντων του διαλύματος περιορίζονται στο ελάχιστο. Αν οι επανειλημμένες εξισορροπήσεις θεωρηθούν ως σειρά ισορροπιών ιονανταλλαγής, εξάγεται μία εξίσωση, ή οποία χρησιμοποιείται για την γραφική απεικόνιση των πειραματικών αποτελεσμάτων υπό γραμμική μορφή. Η μεγίστη

ποσότης τοῦ συγκρατουμένου ἀπὸ τὸ ἔδαφος ἀμμωνίου καὶ ὁ συντελεστὴς τῆς ἰσορροπίας ἰονανταλλαγῆς μποροῦν νὰ προσδιορισθοῦν ἀπὸ τέτοιες γραφικὲς παραστάσεις.

Ἡ μέθοδος ἐφαρμόζεται σὲ διάφορες τάξεις ἐδαφῶν καὶ ἡ ἐπεξεργασία τῶν πειραματικῶν ἀποτελεσμάτων ἀποδεικνύει ὅτι αὐτὰ προσαρμόζονται πρὸς τὴν ἑξαχθεῖσα θεωρητικὴ ἑξίσωση. Οἱ συντελεστὲς ἰσορροπίας καὶ ἡ μεγίστη ἱκανότης συγκρατήσεως τῶν διαφόρων ἐδαφῶν, τὰ ὁποῖα ἐμελετήθησαν, εὐρέθησαν νὰ εἶναι χαρακτηριστικὰ κάθε ἐδαφικῆς τάξεως καὶ ἔτσι συνιστοῦν νέα παράμετρο γιὰ τὴν ἀξιολόγησιν τῶν ἐδαφῶν, ἐν σχέσει μὲ τὴν χρησιμοποίησιν ἀμμωνιακῶν λιπασμάτων.

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SOME MICROSCOPICAL OBSERVATIONS OF GaSe

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Summary

GaSe, grown by the Bridgeman technique, was examined under an electron and an optical microscope. Besides the usual partial dislocations, black rings, very alike to dislocation loops, were attributed to the chloroform used for preparing the specimens. Dendritic growth was observed which was considered to be due to Se degradation by time.

Key words: crystal growth, partial dislocations, dendrite formation.

Introduction

GaSe is one of the most promising materials among the A^{III}B^{VI} group semiconductors. Therefore it has received increasing interest in the past few years. It has very interesting optical properties, an expected band anisotropy and high photosensitivity. It exists in different crystal modifications^{1,2} and is built up of four two dimensional sheets of like atoms in the sequence of Se-Ga-Ga-Se. Strong covalent bonds occur within the multiple layers and only weak van der Waals forces act between neighboring layers. Glide along the basal plane is easy and it occurs between neighboring multiple layers.

Electron microscopy and etch-pit examination have been used to study the microstructure of GaSe. Dislocation networks,^{3,4} growth spirals with either regular or irregular arms on the basal plane^{5,6} and high-angle twins⁷ have been observed.

The aim of the present paper is to present some interesting features of GaSe, which arose during an electron-microscopy study of good single crystals, grown for optical measurements.

CRYSTAL GROWTH

GaSe single crystals were grown using the Bridgeman method, from elements of 99.999% purity manufactured by "Koch-Light". The elements, in stoichiometric proportion, were placed in a quartz tube which was evacuated to about 10^{-5} mmHg and then sealed off. The ampoules were made with double walls. The lower part of the inner tube (either 10 or 13 mm in diameter) had a conical shape about 35 mm long. The ampoule was put in a cold vertical furnace which was then heated up at a rate of about 25° C/hour, until a temperature of about 1000°C was reached. The temperature profile of the furnace is given in Fig. 1. At the start of the growth the ampoule was placed between the points T₁ and T₂. It was held there for several

hours and then it was lowered down into the cooler zone at a speed of about 0.8 mm/hour. The obtained crystals could be easily cleaved into thin plates.

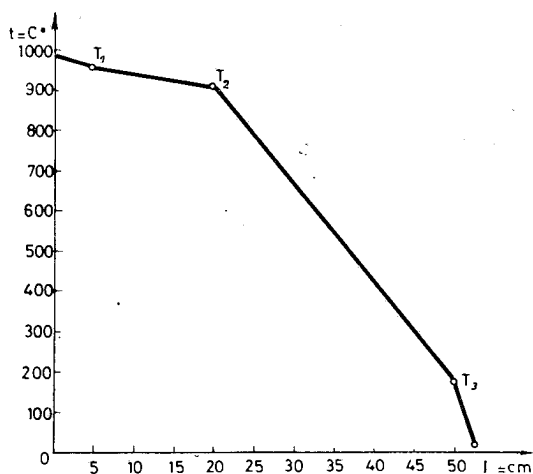


FIG. 1: Profile of the furnace temperature.

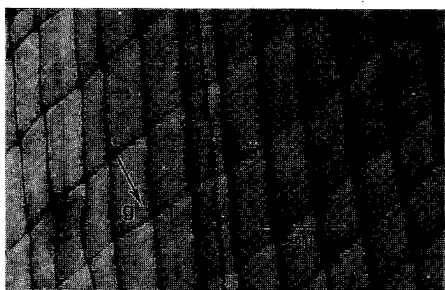


FIG. 2: Grid of partial dislocations in GaSe, typical for a layer-structure material.

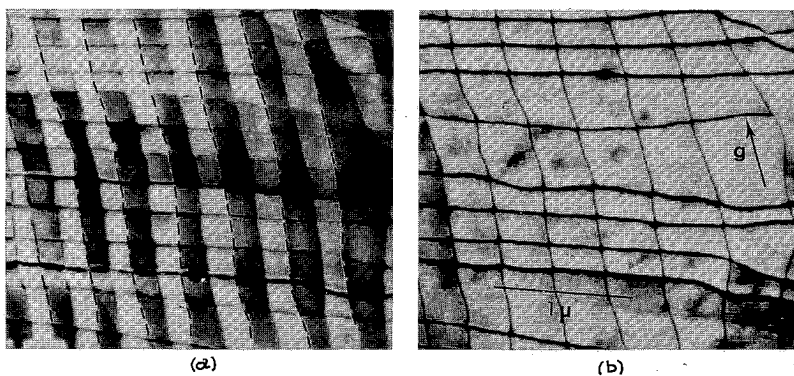


FIG. 3: Micrographs of the same area under different diffraction condition.

(a) Stacking fault contrast.

(b) One set of bounding dislocations, marked by broken lines in (a), is out of contrast. $g = 1210$

Results and discussion

A JEM 100 U electron microscope and a Reichert-Zetopan optical microscope were used for the examination. *Using a cellotape stripe, specimens were obtained by repeated cleavage of the layered structure single crystal. After that, the glue of the stripe was dissolved with chloroform and finally the specimens were fished up on copper grids from a distilled-water bath.*

In the hexagonal close-packed structures (like GaSe) a dissociation of a perfect dislocation into two partials is energetically favourable. These two partials produce a dislocation ribbon and the distance between them (so-called "width of the ribbon") depends on the kind of dislocation and the stacking fault energy γ of the material⁸. When γ is quite small, it is easy to observe such dissociation by usual electron microscopy, otherwise special techniques are needed.⁹ Figure 2 is a typical micrograph of a grid of partial dislocations in GaSe. The distance between vertical partials (one set missing) is of the order of 1200 Å, which means that GaSe is a low stacking fault energy material.

Figure 3 shows another area under different diffraction conditions. Looking at the vertical set, the stacking fault contrast only appears in 3a, while in 3b one set of the bounding dislocations occurs, the second one (marked by broken lines in 3a) being out of contrast. According to the $g \cdot b = 0$ criterion,¹⁰ the Burgers vector of the invisible dislocations must be $1/3 [1010]$. Therefore they are partials in screw orientation.

During the early stages of the investigation, black rings of the order of microns in diameter appeared. Typical examples of these are shown in Figure 4a. The rings affect quite heavily the stress field of the partial dislocations (Fig. 4b), some of which go vertically into the "faulted" region. As in some cases part of a possible dislocation loop (like in A of Fig. 4b) as well as lines of no contrast seemed to appear, we did tilting experiments to examine the case of dislocation loops with an inclined Burgers vector.^{11,12} The results were negative. Stereo pictures showed the existence of a surface step in the region of the black line. Then we examined the crystals under an optical microscope, before and after the preparation of the specimens. The original crystals showed no rings, while these were present in the final specimens, as is obvious in Fig. 5. Therefore, the most reliable explanation for the formation of the rings is that, during the course of dissolution of the cellotape by chloroform, some bubbles are formed with cellotape on their surface. It is this cellotape which forms the rings, when the bubbles break out during the cleaning of the specimen with distilled water. The idea of pre-existing dislocation loops with a $[0001]$ Burgers vector, which had climbed up to the crystal surface, where the steps produced were "decorated" by cellotape, seems rather unlikely (see also Fig. 5).

Finally, some sort of dendritic growth has been observed, mainly near grain boundaries. An example is shown in Figure 6. Besides the main spots of GaSe, some extra spots (such as the ones at A and B) are visible, much easier on the negatives. The questions arose of how these dendrites were formed and what material they were made of.

Figure 7 is a set of micrographs of another area. Fig. 7a and 7b are bright and dark field images respectively and 7c is the corresponding diffraction pattern, correctly oriented. The same kind of extra spots as in Figure 6b are now clearly visible. B-type extra spots are visible also near the 1010 -type main diffraction spots, while a faint diffraction ring, passing over both A and B spots, can be seen. Type A

spots are in the same directions to the main spots but B-type form an angle of 20° with the main diffraction spots of 1120 or 1010-type and their total diffraction constructs another 0001 hexagonal pattern. By indexing this pattern it was found $d = 2,104 \text{ \AA}$, for the new 1120 spots. This value is very near to the corresponding value for Se ($d = 2,184 \text{ \AA}$). Figure 7b was taken using spot E. The symmetrical features of Fig. 7a (marked by C) are not visible on it, while bright areas are clearly visible.

It is well known (see for example refs 13,14) that the origin of the *dendrites* results from the kinetics of crystal growth. *Dendritic* growth results from "competition" for regions which are "favourable", that is regions of higher concentration (in growth from the vapour phase or from solution) or regions of

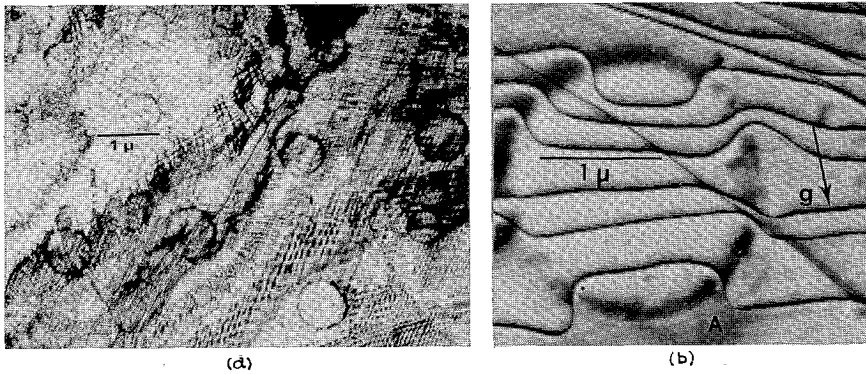


FIG. 4: (a) Micrograph showing black rings in GaSe.

(b) Micrograph showing the reaction of the stress field of the partial dislocations when they approach the ring.

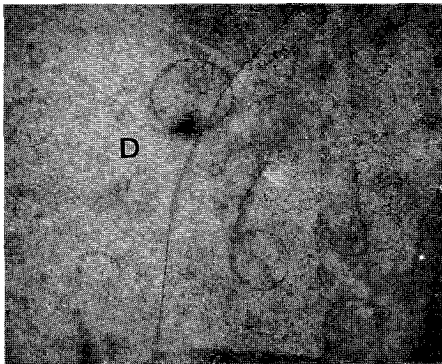


FIG. 5.

Black rings under an optical microscope. Notice that these are formed even over cracks or grain boundaries (ring D).

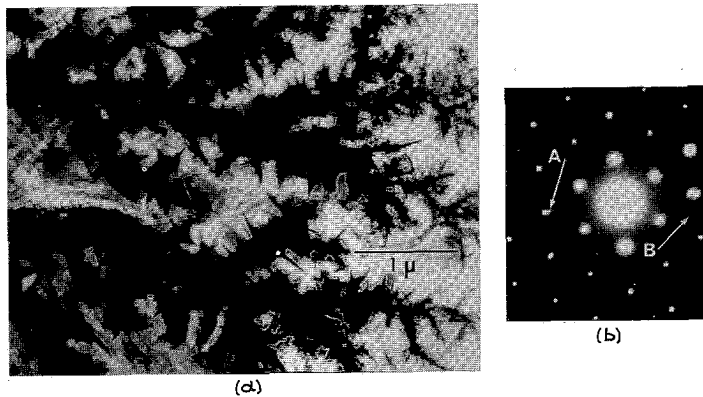


FIG. 6: (a) Dendrite features in GaSe.
(b) The corresponding diffraction pattern (see text for details).

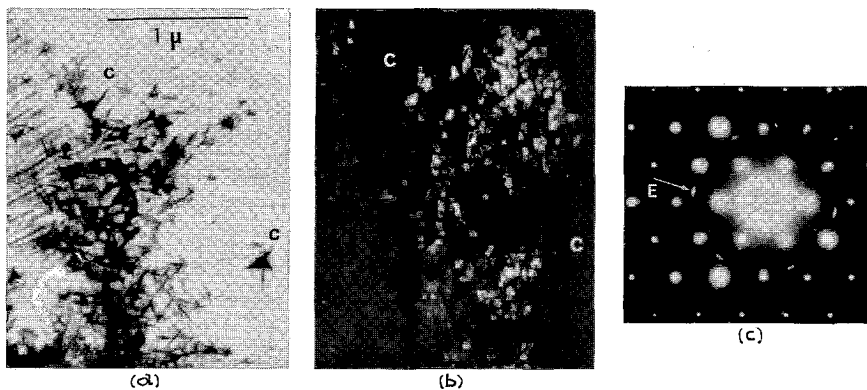


FIG. 7: (a) Bright-field image.
(b) Dark-field image taken using spot E.
(c) The corresponding diffraction pattern (see text for details).

larger undercooling (in growth from the melt). On the other hand, in slow growth there is no shortage of such regions and hence no need for *dendritic* growth. This was the case during our crystal growth (cooling rate 0.8 mm/hour).

From all these data it is concluded that selenium was released and was precipitated in the GaSe matrix with a morphological appearance of dendrites. Recently, during crystallization of amorphous $\text{Ge}_x\text{Se}_{1-x}$ formation of dendrities of pure Se was reported.¹⁵

This formation is more likely to be due to a time degradation rather than to the crystal growth itself. In support of this hypothesis is that Gouskof and Gouskof¹⁶

found different values for hole mobilities in crystals a and b of GaTe. Crystal a was measured five months after its preparation while crystal b was measured immediately after growth.

There are two ways of growth, in both of them selenium has its c-axis parallel to the c-axis of GaSe:

I) Microcrystalline selenium grows with a preferential orientation forming 20° with the corresponding crystallographic direction of GaSe. Due to this, B spots and faint diffraction rings appear on the diffraction pattern of GaSe. Figs. 7b, c support this hypothesis.

II) The selenium atoms grow epitaxially on GaSe, forming a lattice with crystallographic directions parallel to the corresponding ones of GaSe. This leads to the appearance of A spots on the diffraction pattern. Unfortunately, we did not manage to take any dark-field images of these spots. Nevertheless, comparing Figs. 7a and 7c, we can find that the features of 7a, like the ones marked by C, have their sides parallel to the 1010 crystallographic directions of GaSe.

Conclusions

From the material presented, the following conclusions can be drawn:

1) Because of the large distance between the partials, GaSe is a rather small stacking fault energy material.

2) There are no dislocation loops. Black rings, very alike to them, were found to be due to the chloroform used for preparing the specimens.

3) Due to a time degradation, selenium is released, which precipitates in the GaSe matrix in two ways: epitaxially, with its axes parallel to the corresponding ones of GaSe or as microcrystalline Se, with the c-axis parallel to the c-axis of GaSe, but having a and b axes preferentially forming 20° with the corresponding ones of GaSe.

ΠΕΡΙΛΗΨΗ

Μικροσκοπικές παρατηρήσεις σε GaSe.

Κρύσταλλοι GaSe (έξαγωνικής δομής), που αναπτύχθηκαν με τη μέθοδο Bridgeman, εξετάστηκαν με ηλεκτρονικό και οπτικό μικροσκόπιο. Οί κλασματικές έξαρμόσεις, που παρατηρήθηκαν, είχαν μεταξύ τους μια απόσταση μεγαλύτερη από 1000 Å, πράγμα που δείχνει ότι το GaSe είναι υλικό με μικρή ενέργεια σχηματισμού σφαλμάτων έπιστοιβάσης. Επίσης παρατηρήθηκαν μαύροι κύκλοι, που ή συμπεριφορά τους έμοιαζε με τη συμπεριφορά κυκλικών έξαρμόσεων. Τελικά όμως βρέθηκε ότι ήταν επιφανειακό φαινόμενο και όφειλόνταν σε cello tape που παρέμενε στην επιφάνεια ύστερα από τη διάρρηξη φυσαλίδων χλωροφορμίου, στο στάδιο της παρασκευής τών δειγμάτων. Τέλος παρατηρήθηκε σχηματισμός δενδριτών, οί όποίοι αποδόθηκαν στο ότι το GaSe, με το πέρασμα του χρόνου, χάνει Se. Το Se αυτό κάθεται στο πλέγμα του GaSe κατά δύο τρόπους, έχοντας και στους δύο έξαγωνική δομή: είτε αναπτύσσεται έπιταξιακά με κρυσταλλογραφικούς άξονες άκριβώς παράλληλους με τους αντίστοιχους του GaSe είτε σχηματίζει μικροκρυσταλλικό Se, με μία προτιμητέα κατάσταση όπου οί άξονές του σχηματίζουν 20° με τους αντίστοιχους του GaSe.

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