Solubility was studied by stirring a solution of the protein at the appropriate pH value or concentration of acid for 15 min. Then the suspension was centrifuged at 8000 rpm for 10 min. An aliquot was taken from the supernatant liquid and its protein content was determined by the biuret method.

The degrees of denaturation of the separated fractions were determined by a polarographic method on a LP-7 polarograph [8].

Phytin contents were determined as described by Tevekelov [9].

SUMMARY

1. It has been shown that protein isolates of the cotton plant obtained by extraction in an acid medium contain mainly globulins.

2. It has been established that the presence of phytin, which is strongly bound to proteins in an acid medium, affects the properties of the globulins.

LITERATURE CITED

1. US Patent No. 3,615,657 (1971).
2. US Patent No. 3,124,461 (1964).
3. E. W. Lucas, J. T. Jawhon, K. C. Rhee, and R. G. Simmons, J. Am. Oil Chemists' Soc., 58, No. 7, 581 (1981).
4. A. K. Smith and J. J. Rackis, J. Am. Chem.,Soc., 79, 633 (1957).
5. K. Okubo, D. V. Myers, and G. A. Jacobucci, Cereal Chem., 53, 513 (1976).
6. R. Barre, J. E. Courtois, P. Delrien, and R. Peries, Ann. Pharm. Fr., 12, 601 (1954).
7. T. S. Yunusov, M. T. Turakhozhaev, E. G. Yadgarov, and T. T. Shakirov, Khim. Prir. Soedin., 473 (1981).
8. S. I. Asatov, T. S. Yunusov, and P. Kh. Yuldashev, Khim. Prir. Soedin., 800 (1980).
9. D. Tevekelov, Izv. Inst. Khranene Bolg. Akad. Nauk 7, 21 (1968).

ONE-STAGE SYNTHESIS OF INDANONES IN THE BENZIMIDAZOLIN-2-ONE SERIES

Ch. Sh. Kadyrov and S. S. Khalikov

UDC 547.783+542.91/632.952

The possibility has been shown of a one-stage synthesis of 5,6-ureylene-3-methylindan-1-one and 6,7-ureylene-3,5-dimethylindan-1-one by condensing the corresponding benzimidazolin-2-ones with γ-butyrolactone or crotonic acid in the presence of aluminum chloride. The reaction was performed at a ratio of the reactants benzimidazolin-2-one (5-methylbenzimidazolin-2-one):γ-butyrolactone (crotonic acid):$AlCl_3$ = 1:1:6. Several derivatives (oximes, semicarbazones, substituted hydrazones) have been obtained that confirm the ketonic structures of the indanones synthesized. The plant growth inhibiting and fungicidal activities of the compounds synthesized have been studied.

Recently, various natural indanones possessing biological activity have been isolated from plant objects [1]. At the same time, polycyclic indanones exhibit the properties of plant growth inhibitors [2], and such ketone derivatives as semicarbazones, thiosemicarbazones, and hydrazones possess fungicidal activity [3]. We have previously synthesized 5,6-ureylene-3-methylindan-1-one (V) and 6,7-ureylene-3,5-dimethylindan-1-one (VI) by the cyclization of the corresponding 5-(2)carboxy-1-methylethyl)- and 5-(2-carboxy-1-methylethyl)-6-methylbenzimidazolin-2-ones (III) and (IV) in concentrated sulfuric acid [4].

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 369-373, May-June, 1983. Original article submitted May 12, 1982.

 0009-3130/83/1903-0348 $07.50

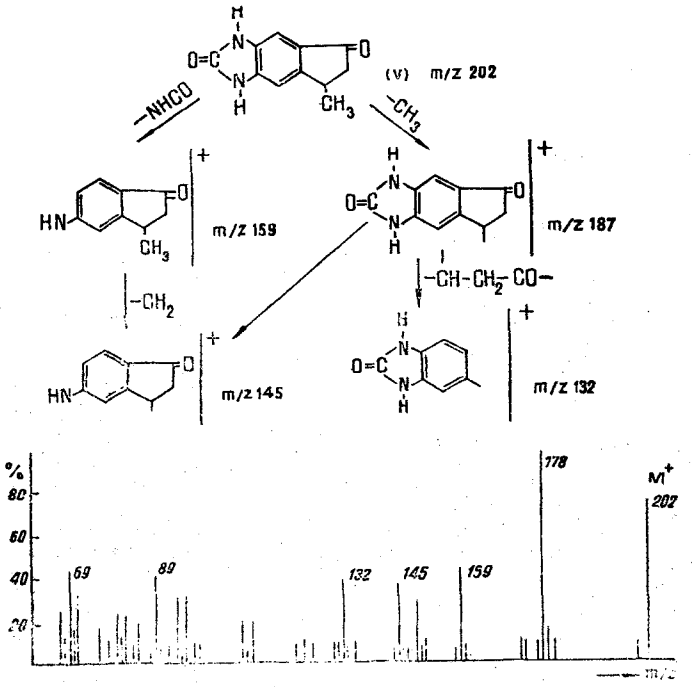
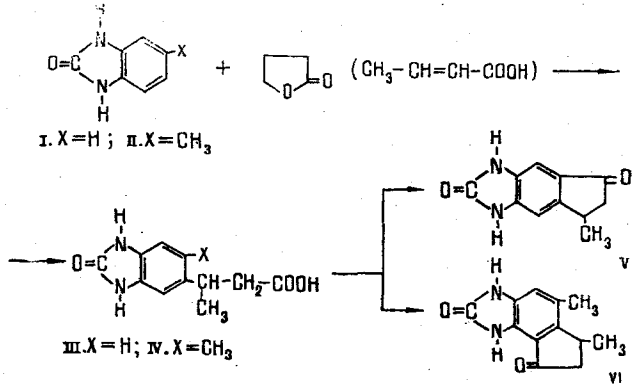


Fig. 1. Mass spectrum of (V).

In order to study the inhibiting properties of the indanones (V) and (VI) and also the fungicidal properties of derivatives of (V) and (VI), we have now synthesized (V) and (VI) from the corresponding (2-carboxy-1-methylethyl)benzimidazolin-2-ones (III) and (IV), which, in their turn, were obtained by condensing benzimidazolin-2-one (I) and 5-methylbenzimidazolin-2-one (II) with γ-butyrolactone (or crotonic acid), in the presence of anhydrous aluminum chloride. Simultaneously with this, we have developed a one-stage synthesis of (V) and (VI) by condensing (I) and (II) with γ-butyrolactone (or crotonic acid) in the presence of an excess of $AlCl_3$. The reaction was performed at a ratio of the initial reactants (I) (or (II)): γ-butyrolactone (crotonic acid): $AlCl_3$ of 1:1:6 in tetrachloroethylene with heating at 120-125°C for 6 h.

An analogous cyclization has been reported in a handbook [5], where it is stated that in the reactions of benzene with certain carboxylic acids in the presence of an excess of $AlCl_3$ the corresponding indanones and tetralones are formed.

The formation of (V) and (VI) takes place in accordance with the following scheme:



The structures of the indanones (V) and (VI) obtained were shown by IR, UV, and mass spectroscopy.

The IR spectrum of (V) has two absorption bands in the 855-870 cm^{-1} region corresponding to a 1,2,4,5-substituted aromatic ring, while two absorption bands somewhat remote from one another are observed for (VI) – one of them an intense band at 1750 cm^{-1} and the other less intense at 1920 cm^{-1} – which are characteristic for 1,2,3,4,5-substituted aromatics [6].

The UV spectra of (V) and (VI) each have four maxima, three of them being characteristic for the ab-

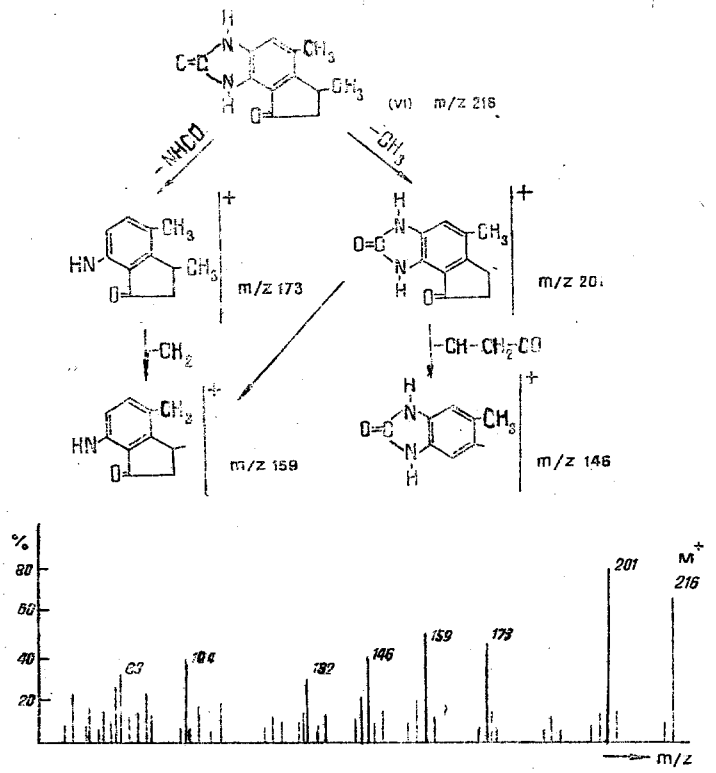


Fig. 2. Mass spectrum of (VI).

sorption of a benzimidazolone ring (λ 210-220 nm, λ 230-240 nm, λ 280-290 nm) and the fourth maximum (λ 320-350 nm) arising from the conjugation of the carbonyl group formed on cyclization with the aromatic ring, i.e., this maximum characterizes a conjugated carbonyl group.

The mass spectra of (V) and (VI) were characterized by the presence of the corresponding peaks of the molecular ions with m/z 202 and 216, and also by the peaks of ions with m/z 187 and 201, 159 and 173, 145 and 159, and 132 and 146, respectively.

On the basis of the mass spectra of (V) and (VI) their fragmentation pathways can be represented in the manner shown in Figs. 1 and 2.

The ketonic structures of compound (V) and (VI) were confirmed by the performance of the characteristic reactions of ketones with hydroxylamine, semicarbazide, and 2,4-dinitrophenylhydrazine. These reactions gave the corresponding oximes, semicarbazones, and 2,4-dinitrophenylhydrazones of (V) and (VI).

The inhibitory activities of (V) and (VI) were studied on the growth of coleoptiles of the wheat variety Albidium 43 at concentrations of $1.0 \cdot 10^{-2}$–$1.0 \cdot 10^{-4}$%. It was established that compound (V) possesses inhibitory activity in comparison with the control (2% sucrose), and in comparison with a standard (sodium salt of 2,3,5-triiodobenzoic acid) it showed a weak inhibitory effect. Compound (VI) in a concentration of $1.0 \cdot 10^{-3}$–$1.0 \cdot 10^{-4}$% was superior to the standard in its inhibitory effect by 17-24%. Thus, as we see, the introduction of a methyl group into an indanone leads to an increase in its inhibitory effect.

The derivatives (oximes, semicarbazones, 2,4-dinitrophenylhydrazones) of compounds (V) and (VI) showed weak fungicidal activities.

The results of a study of the fungicidal activities of the compounds synthesized showed that the efficacy of the benzimidazolones rises with the introduction of electronegative groups into the aromatic nucleus (C – at the level of the control, the control being distilled water) (see table on following page). Thus, the indanones (V) and (VI), which are cyclic ketone derivatives of compounds (I) and (II) possess higher activities than (I) and (II), respectively, while the introduction of a methyl group (electron donor) or of more complicated alkyl groups (carboxypropyl radical) in position 5 increases the activities as compared with (I) and (II). In the series of indanones (V) and (VI) the fungicidal activity for all four test fungi rose on the introduction of the methyl group, possibly because of a change in the skeleton of the indanone. Derivatives of the indanone (V) at the carbonyl group (semicarbazone, 2,4-dinitrophenylhydrazone, and oxime) are more active fungicides than the indanone (V) itself. It is known [3] that oximes are active antisporulants.

Name of the substance	*Verticillium dahliae*	*Fusarium oxysporium*	*Thielaviopsis basicola*	*Rhizoctonia solani*
Benzimidazolin-2-one (I)	26	18	38	30
5-Methylbenzimidazolin-2-one (II)	7	C	C	C
5-Chlorobenzimidazolin-2-one	47	41		62
5-(β-Carboxy-1-methylethyl)-benzimidazolin-2-one (III)	10	C	C	20
5-(β-Carboxy-1-methylethyl)-6-methylbenzimidazolin-2-one (IV)	C	6	22	24
5,6-Ureylene-3-methylindan-1-one (V)	35	16	2	9
6,7-Ureylene-3,5-dimethyl-indan-1-one (VI)	57	18	60	68
Semicarbazone of (V)	39	C	45	42
2,4-Dinitrophenylhydrazone of (V)	48	38	1	29
Oxime of (V)	36	22	32	25

EXPERIMENTAL

IR spectra were taken on a UR-20 spectrometer in tablets molded with KBr, UV spectra on a Hitachi EPS-3T spectrometer (with ethanol as solvent), and mass spectra on a MKh-303 mass spectrometer. The results of elementary analysis for the derivatives of (V) and (VI) corresponded to the calculated figures.

5,6-Ureylene-2-methylindan-1-one [7-Methyl-2,3,5,6-tetrahydro-1H,8H-cyclopenta[f]benzimidazole-2,5-dione] (V). With ice cooling and vigorous stirring, 80.0 g (0.6 mole) of anhydrous $AlCl_3$ was added in portions to a suspension of 13.5 g (0.1 mole) of (I) and 8.6 g (0.1 mole) of γ-butyrolactone (or crotonic acid) in 500 ml of tetrachloroethylene. Then the reaction mixture was heated at 120-125°C for 6 h, whereupon gaseous hydrogen chloride was gradually evolved. After cooling, the reaction mixture was hydrolyzed with a mixture of 100 ml of hydrochloric acid (d 1.49) and ice, and the tetrachloroethylene was distilled off with steam. From the acidic residual solution a black oily precipitate deposited, which was filtered off, and the filtrate on standing deposited a yellowish precipitate of (V). Recrystallization from aqueous ethanol gave colorless crystals with mp 318-320°C. Yield 39%, R_f 0.66 (propan-2-ol–ammonia–ethyl acetate (1:3:6) system). By three successive treatments with sodium carbonate, the black oily precipitate gave (III) with mp 250-252°C, yield, 16%, R_f 0.28 (same system).

6,7-Ureylene-2,5-dimethylindan-1-one [5,6-Dimethyl-2,3,5,6-tetrahydro-1H,8H-cyclopenta[e]benzimidazole-2,8-dione] (VI). In a three-necked flask fitted with a stirrer and reflux condenser, 80.0 g (0.6 mole) of anhydrous $AlCl_3$ was added in small portions with ice cooling and vigorous stirring to a suspension of 14.8 g (0.1 mole) of (II) and 8.6 g (0.1 mole) of γ-butyrolactone (or crotonic acid). The subsequent working up was similar to that described above. Colorless crystals of (VI) were obtained with mp 271-272°C, yield 28%, R_f 0.68 (propan-2-ol–ammonia–ethyl acetate (1:3:6) system) and (IV) with mp 241-242°C, yield 28% (R_f 0.30 (same system).

The oximes, semicarbazones, and 2,4-dinitrophenylhydrazones of (V) and (VI) were obtained as described in a handbook [7].

Oxime of 5,6-Ureylene-3-methylindan-1-one. Colorless crystals, mp 295-297°C (aqueous ethanol), R_f 0.52.

Oxime of 6,7-Ureylene-3,5-dimethylindan-1-one. Colorless crystals, Yield 48%, mp 283-285°C (aqueous ethanol), R_f 0.50.

Semicarbazone of 5,6-Ureylene-3-methylindan-1-one. Colorless crystals. Yield 90%, mp 300°C (decomp), R_f 0.63.

Semicarbazone of 6,7-Ureylene-3,5-dimethyl-indan-1-one. Colorless crystals. Yield 82%, mp 280°C (decomp), R_f 0.65.

2,4-Dinitrophenylhydrazone of 5,6-Ureylene-3-methyl-indan-1-one. Amorphous red crystals. Yield 92% mp 260°C (decomp.), R_f 0.35.

2,4-Dinitrophenylhydrazone of 6,7-Ureylene-3,5-dimethylindan-1-one. Amorphous red crystals. Yield 80%, mp 235°C (decomp.), R_f 0.37.

The individualities of the derivatives of the indanones (V) and (VI) were checked by the TLC method (Silufol UV-254; visualizing agent 1% $KMnO_4$ in 4% aqueous H_2SO_4). Solvent systems: for the oximes, propan-2-ol–ammonia–ethyl acetate (1:3:6); for the semicarbazones, propan-2-ol–ethyl acetate (1:4): and for the 2,4-dinitrophenylhydrazones, benzene–acetone (3:2).

SUMMARY

1. The possibility has been shown of obtaining 5,6-ureylene-3-methylindan-1-one and 6,7-ureylene-3,5-dimethylindan-1-one in one stage by condensing the corresponding benzimidazolin-2-ones with γ-butyrolactone or crotonic acid in the presence of an excess of anhydrous aluminum chloride.

2. The plant growth inhibiting activity of the indanones synthesized has been studied.

3. Derivatives of the indanones with hydroxylamine, semicarbazide, and 2,4-dinitrophenylhydrazine have been obtained and their fungicidal activities have been studied.

LITERATURE CITED

1. A. I. Syrchina and A. A. Semenov, Khim. Prir. Soedin., 3 (1982).
2. G. F. Katekar and A. E. Geissler, Plant Physiol., 60, 826 (1977).
3. I. G. Khorsvoll, Abstracts of the 8th International Congress on Plant Protection [in Russian], Moscow (1975).
4. Ch. Sh. Kadyrov, M. N. Kosyakovskaya, and M. R. Yagudaev, Uzb. Khim. Zh., No. 3, 39 (1969).
5. D. Cram and G. S. Hammond, Organic Chemistry, McGraw-Hill, New York (1959).
6. L. A. Kazitsyn and N. B. Kupletskaya, The Use of UV, IR, NMR, and Mass Spectroscopy in Organic Chemistry [in Russian], Moscow (1979).
7. N. V. Vasil'eva, N. B. Kupletskaya, and T. A. Smolina, Practical Work in Organic Chemistry [in Russian], Moscow (1978), p. 167.

ISOLATION OF A LIPASE INHIBITOR FROM THE FUNGUS *Rhizopus microsporus*

K. Davranov, Z. R. Akhmedova, and A. M. Bezborodov

UDC 577.153.2

A factor capable of causing inhibition of the activity of extracellular lipase has been detected in the mycelium of the fungus Rhizopus microsporus. By fractionating a homogenate of the mycelium followed by chromatographic purification on a column of DEAE-cellulose and on Sephadex G-75 this inhibitor has been isolated in the electrophoretically homogeneous state. It is a substance of protein nature with M ~ 24,000, consisting of two subunits. The inhibitor acts on the isoenzymes of the lipase to different extents.

The fungus Rhizopus microsporus is known as a producing agent of active lipases. On studying various components, we detected a protein factor capable of exerting an inhibiting action on extracellular lipase.

Figure 1A presents the results of gel filtration of the supernatant liquid from a homogenate on a column of Sephadex G-75. When the influence of the individual fractions on the activity of the extracellular lipase the preparation of which has been described previously [1] was studied, it was found that elution fractions 42-46 contained a protein causing inhibition (Fig. 1A). These fractions were combined, dialyzed against distilled water, and freeze-dried. The resulting preparation was subjected to ion-exchange chromatography on a column (1× 15 cm) of DEAE-cellulose in 0.01 M phosphate-citrate buffer, pH 7.4. The proteins were eluted with a linear gradient of NaCl from 0 to 1 M at the rate of 12 ml/h. Fractions with a volume of 3 ml were collected (Fig. 1B). Of the five protein peaks obtained, only the proteins of fractions 51-56 possessed inhibitory capability.

A repeat of gel filtration on Sephadex G-75, after dialysis and lyophilization, permitted us to obtain the lipase inhibitor in an electrophoretically homogeneous state (Fig. 1B). The homogeneity of the inhibitor was shown by rechromatography (ion-exchange chromatography on DEAE-cellulose – the protein eluted as one peak

Institute of Microbiology, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 373-375, May-June, 1983. Original article submitted June 3, 1982.

 0009-3130/83/1903-0352 $07.50