BENZOXAZOLINONES.

VI. REACTION OF BENZOXAZOLINONE AND OF BENZOAZOLINETHIONE WITH SUBSTITUTED α-HALO CARBONYL COMPOUNDS

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The alkylation of the potassium and triethylammonium salts of benzoxazolinones and benzoxazolinethiones with α-halo carbonyl compounds has been studied. The reaction takes place at the less electronegiative atoms of the ambident benzoxazolinone and benzoxazolinethione anions. On the nitration of 3-phenacylbenzoxazoline moiety of the molecule.

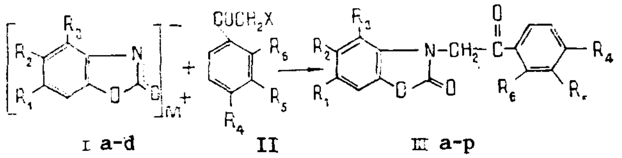
Benzoxazolinone and its methoxy analog are substances of plant origin. Chin-Ming Chen et al. isolated 6-methoxybenzoxazolinone from a dry powder of the roots of *Coix lacryma Jobi* L. and *Scoparia dulcus* [1, 2]. In small doses, benzoxazolinone stimulates, and in larger doses it inhibits, the growth of the seeds of certain plants [3]. Thanks to its presence in rye and maize roots the latter exhibit resistance to a number of diseases [4]. There is information according to which benzoxazolinone is a phytoalexin [5].

With the aim of synthesizing new plant growth regulators we have investigated the reactions of benzoxazolinone (BN) and of benzoxazolinonethione (BT) and their derivatives with α-halo carbonyl compounds.

On the interaction of the triethylammonium salt of benzoxazolinone (Ia, M = $NH(C_2H_{53})$) with phenacyl chloride (IIa) in benzene at 50-60°C for 8 h, 3-phenacylbenzoxazolinone (IIIa) was formed (method A).

The mass spectrum of this compound had the peak of the molecular ion with m/z 253 and of fragments 148 ($M^+ - COC_6H_5$) and 134 ($M^+ - CH_2COC_6H_5$), and in the IR spectrum there were the absorption bands at the stretching vibrations of carbonyl groups in the 1780 and 1680 cm^{-1} regions.

On the basis of these facts, it may be concluded that in the reaction [6] of the benzoxazolinones with phenacyl chlorides the main reaction center is the less electronegative, more polarizable, center of the ambident system — the nitrogen atom.



I a-d II III a-p

$M = K,\ NH(C_2H_5)_3$

III. a. $R_1=R_2=R_3=R_4=R_5=R_6=H$
b. $R_1=Cl,\ R_2=R_3=R_4=R_5=R_6=H$
c. $R_1=Br,\ R_2=R_3=R_4=R_5=R_6=H$
d. $R_1=NO_2,\ R_2=R_3=R_4=R_5=R_6=H$
e. $R_1=R_2=R_3=H,\ R_4=CH_2,\ R_5=R_6=H$
f. $R_1=R_2=R_3=H,\ R_4=C_2H_5,\ R_5=R_6=H$
g. $R_1=R_2=R_3=H,\ R_4=Cl,\ R_5=R_6=H$
h. $R_1=R_2=R_3=H,\ R_4=CH_3,\ R_5=Cl,\ R_6=H$
i. $R_1=R_2=R_3=H,\ R_4=Cl,\ R_5=H,\ R_6=CH_3$
j. $R_1=R_2=R_3=H,\ R_4=CH_3,\ R_5=H,\ R_6=CH_3$
k. $R_1=NO_2,\ R_2=R_3=H,\ R_4=CH_3,\ R_5=R_6=H$
l. $R_1=NO_2,\ R_2=NO_2,\ R_3=R_4=R_5=R_6=H$

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m. $R_1=Cl$, $R_2=NO_2$, $R_3=R_4=R_5=R_6=H$
n. $R_1=R_2=R_3=H$, $R_4=NO_2$, $R_5=R_6=H$
o. $R_1=Cl$, $R_2=R_3=H$, $R_4=NO_2$, $R_5=R_6=H$
p. $R_1=Br$, $R_2=R_3=H$, $R_4=NO_2$, $R_5=R_6=H$

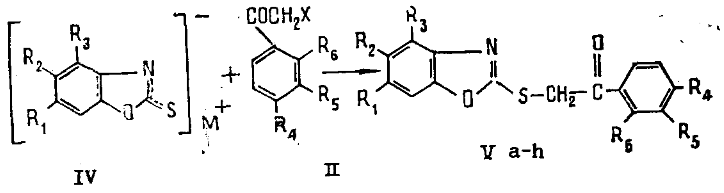
The reaction of the potassium salt of benzoxazolinone with phenacyl chloride in an aqueous medium at 50-60,C for 8 h did not lead to a positive result, but a change in the conditions (85-90°C, 20 h) enabled 3-phenacylbenzoxazolinone to be obtained (method B). The maximum yield of compound (IIIa) (82%) was recorded when the reaction was performed in an ethanolic-alkaline medium (method B, Table 1). The optimum conditions for the alkalyation of benzoxazolinone were: ratio of the reactants BN to phenacyl chloride 1:1.2; temperature 90-95°C; time 2 h. Substituted phenacyl chlorides also alkylated benzoxazolinones (see Table 1). The introduction of electronegative atoms (Cl or Br) into position 6 of benzoxazolinone lowered the yields of 3-phenacylbenzoxazolinones.

When 6-nitrobenzoxazolinone (Id) was alkylated with phenacyl chloride (IIa) (method B) 6-nitro-3-phenacylbenzoxazolinone (IIId) was formed, which was also obtained by the nitration of 3-phenacylbenzoxazolinone (IIIa) with nitric acid. When compound (IIIe) was nitrated, 3-(4-methylphenacyl)-6-nitrobenzoxazolinone (IIIk) was obtained. The structure of the benzoxazolinone (IIId) was confirmed by its mass spectrum, which had the peak of the molecular ion with m/z 298 and fragments with m/z 193 ($M - COC_6H_5$) and 179 ($M - CH_2COC_6H_5$).

When 6-chloro-3-phenacylbenzoxazolinone (IIIb) was nitrated with nitric acid, 6-chloro-5-nitro-3-phenacylbenzoxazolinone (IIIm) was obtained. The mass spectrum of the latter had the peak of the molecular ion with m/z 332 and fragments 227 ($M - COC_6H_5$) and 213 ($M - CH_2COC_6H_5$), and its IR spectrum contained absorption bands at 1690 cm^{-1} ($\nu C{=}O$ in ketones) and 1780 cm^{-1} ($\nu C{=}O$ in benzoxazolinones).

To compare the reactivities of the nitrophenacyl halides with the phenacyl halides in the alkylation of BN and BT, it appeared of interest to synthesize an alkylating agent containing a nitro group in the aromatic ring. However, it was impossible to obtain the latter by acylating nitrobenzene under the conditions of the Friedel—Crafts reaction, and an attempt to nitrate phenacyl chloride was likewise unsuccessful. The necessary p-nitrophenacyl bromide was synthesized by the nitration of ethylbenzene followed by the oxidation of the product to p-nitroacetophenone and the bromination of the latter [7]. The reactions of the benzoxazolinones (Ia-c) with p-nitrophenacyl bromides were performed under the conditions developed for alkylation by phenacyl chlorides. As can be seen from Table 1, the yields of products (IIIn-p) were low.

The benzoxazolinonethiones (IV) were alkylated with α-halo carbonyl compounds under the same conditions (see Table 1).



$M=K$, $NH(C_2H_5)_3$

Va. $R_1=R_2=R_3=R_4=R_5=R_6=H$
b. $R_1=R_2=R_3=H$, $R_4=CH_3$, $R_5=R_6=H$
c. $R_1=R_2=R_3=H$, $R_4=C_2H_5$, $R_5=R_6=H$
d. $R_1=R_2=R_3=H$, $R_4=CH_3$, $R_5=H$, $R_6=CH_3$
e. $R_1=R_2=R_3=H$, $R_4=Cl$, $R_5=H$, $R_6=CH_3$
f. $R_1=R_2=R_3=H$, $R_4=CH_3$, $R_5=Cl$, $R_6=H$
g. $R_1=R_2=R_3=H$, $R_4=Cl$, $R_5=R_6=H$
h. $R_1=NO_2$, $R_2=R_3=R_4=R_5=R_6=H$

TABLE 1. Properties of the Compounds Synthesized

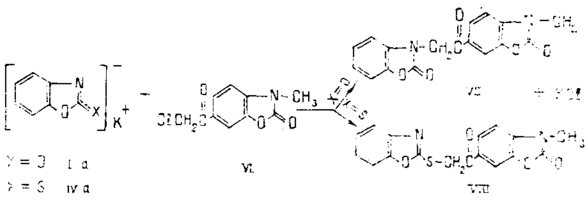
Compound	Method	Yield, %	mp, °C (ethanol)	R_f	M^+	Empirical formula
			3-Phenacylbenzoxasolinones			
III a	C	82	133—135	0,65	253	$C_{15}H_{11}NO_3$
III b	A	15	173—175	0,56	287	$C_{15}H_{10}ClNO_3$
III c	C	42	181—183	0,86	—	$C_{16}H_{10}BrNO_5$
III d	C	54	211—212	0,58	298	$C_{15}H_{10}NO_5$
III e	C	58	146—148*	0,81	267	$C_{16}H_{13}NO_3$
III f	C	41	119—121**	0,72	281	$C_{17}H_{15}NO_3$
III g	C	61	177—178	0,75	—	$C_{15}H_{10}ClNO_3$
III h	A	33	150—152	0,75	301	$C_{16}H_{12}ClNO_3$
III i	A	25	163—165	0,81	301	$C_{16}H_{12}ClNO_3$
III j	A	28	112—114	0,73	281	$C_{17}H_{15}NO_3$
III k	—	44	210—212	0,68	343	$C_{15}H_{10}N_3O_7$
III l	—	13	152—153**	0,69	312	$C_{16}H_{12}N_2O_5$
III m	—	57	202—204	0,81	332	$C_{15}H_9ClN_2O_5$
III n	A	40	212—214***	0, 78	298	$C_{15}H_{10}N_2O_5$
III o	A	39	203—205	—	332	$C_{15}H_9ClN_2O_5$
III p	A	28	226—228	—	377	$C_{15}H_9BrN_2O_5$
			2-(Phenacylthio) benzoxazoles			
Va	A	80	111—113	269	0,88	$C_{15}H_{11}NO_2S$
V b	B	89	110—112	283	0,95	$C_{16}H_{13}NO_2S$
V c	B	75	96—98	297	0,89	$C_{17}H_{15}NO_3S$
V d	A	70	92—93	297	0,78	$C_{17}H_{15}NO_2S$
V e	A	75	95—96	317	0,84	$C_{16}H_{17}NO_2S$
V f	A	53	112—114	317	0,90	$C_{16}H_{12}ClNO_2S$
V g	A	85	118—120	303	0,85	$C_{15}H_{10}ClNO_2S$
V h	C	25	167—169*	314	—	$C_{15}H_{10}N_2O_4S$

*From benzene—hexane (1:1)
**From benzene
***From acetone

According to the UV spectrum of (Vk) (λ_{max} 288 nm, log ε 4.6), the reaction of (IV) with phenacyl chlorides, as with other alkylating agents [8], formed S-substituted products.

The mass spectrum of compound (Va) had the peak of the molecular ion with m/z 269, and also fragments 241 (M — CO), 227 (M — CH_2CO), 164 (M — COC_6H_5), and 150 (M — $CH_2CO_6H_5$). The IR spectra of (Va) contained an absorption band at 1680 cm^{-1}.

The presence of a mobile halogen atom in the molecule of 6-chloroacetyl-3-methylbenzoxazolinone (VI, mp 155-157°C) served as a basis for using the latter as an alkylating agent and comparing its reactivity with that of phenacyl chloride. When the reaction of the benzoxazolinone (Ia) with compound (VI) was performed by method B, 3-methyl-6-(2'-oxobenzoxazol-3'-ylacetyl)benzoxalinone was obtained. Judging from the yields of reaction products (82 and 28%, respectively), phenacyl chloride is more reactive than 6-chloroacetyl-3-methylbenzoxazolinone.



The reaction of the potassium salt of benzoxazolinethione with 6-chloroacetyl-3-methylbenzoxazolinone (VI) in an aqueous medium for 2 h at room temperature formed 6-(benzoxazol-2'-thioacetyl-3-methylbenzoxazolinone (VIII).

The compound synthesized exhibited moderate fungicidal activity.

EXPERIMENTAL

IR spectra were taken on a UR-10 spectrophotometer (KBr), UV spectra on a Hitachi-3T spectrophotometer, and mass spectra on a MKh-1303 instrument at 100°C and an energy of 40 eV. The purities of the compounds synthesized were checked on Silufol plates in the benzene—ethanol

(21:2) system with a mixture of 48 ml of H_2O, 0.5 g of $KMnO_4$, and 2 ml of H_2SO_4 as the revealing agent. The results of the analysis of all the compounds corresponded to the calculated figures.

3-Phenacylbenzoxazolinone (IIIa). A. With stirring, 3.08 g (20 mmole) of phenacyl chloride in 20 ml of dry benzene was added dropwise to a mixture of 2.7 g (20 mmole) of benzoxazolinone and 2.02 g (20 mmole) of TEA in 150 ml of dry benzene. The mixture was heated in the water bath at 50-60°C for 8 h. The precipitate of TEA hydrochloride was filtered off. After evaporation of the benzene solution, 2.39 g (47%) of compound (IIIa) was obtained. IR spectrum, cm^{-1}: 1810 and 1680.

B. With stirring, 1.54 g (10 mmole) of phenacyl chloride dissolved in 10 ml of acetone was added to a mixture of 1.35 g (10 mmole) of compounds (Ia) and 0.56 g (10 mmole) of KOH in 20 ml of water. The mixture was heated at 50-60°C for 7 h and at 85-90°C for 20 h. The resinous precipitate that formed was extracted with benzene. Evaporation of the benzene solution gave 24% of product completely identical with that described in paragraph A.

C. A mixture of 1.35 g (10 mmole) of the compound BN, 0.66 g (12 mmole) of KOH, and 3 ml of water was treated with 1.38 g (12 mmole) of phenacyl chloride dissolved in 55 ml of ethanol. The mixture was heated at 90-95°C for 2 h. The precipitate that deposited was separated off by filtration and was washed with water and dried. This gave 1.66 g (82%) of a product completely identical with that described in paragraph A. The benzoxazolinones (IIIb-p) were obtained similarly (see Table 1).

6-Nitro-3-phenacylbenzoxazolinone (IIId). With stirring, 6 ml of nitric acid (d = 1.42) was added to 1.72 g of compound (IIIa), and the mixture was heated at 45-50°C for 30 min. After the treatment of the mixture with water, the precipitate that had deposited was filtered off to give 0.41 g (28%) of a product completely identical with that obtained by method C (see Table).

6-Chloro-5-nitro-3-phenacylbenzoxazolinone (IIIm). With ice cooling and stirring, 6 ml of nitric acid (d = 1.42) was added dropwise to 2.87 g (10 mmole) of compound (IIIb). Then the mixture was stirred at room temperature for 1 h and was heated in the water bath at 50-60°C for 30 min. After the reaction mixture had been treated with ice and the resulting precipitate had been separated off, 1.89 g of 6-chloro-5-nitro-3-phenacylbenzoxazolinone (IIIm) was obtained. 3-(4-Methylphenacyl)-6-nitrobenzoxazolinone (IIIk) was obtained similarly (see Table 1).

The 2-(phenacylthio)benzoxazoles (Va-h) were obtained similarly to the nitrophenacylbenzoxazolinone (IIIa-m) by methods A, B, and C (see Table 1).

3-Methyl-6-(2'-oxobenzoxazol-3'-ylacetyl)benzoxazolinone (VII) was obtained by method B from 0.76 g (5 mmole) of compound (Ia), 0.33 g (6 mmole) of KOH, and 1.75 g (6 mmole) of 6-chloroacetyl-3-methylbenzoxazolinone (VI). Yield 0.41 g (28%), mp 236-239°C (acetone).

6-(Benzoxazol-2'-thioacetyl)-3-methylbenzoxalinone (VIII). This was obtained by method B from 0.75 g (5 mmole) of compound (IV), 0.39 g (6 mmole) of KOH, and 1.7 g (5 mmole) of 6-chloroacetyl-3-methylbenzoxazolinone. Yield 0.42 g (44%), mp 177-179°C (acetone). Mass spectrum, m/z (%): M^+ 340 (36), 312 (97), 206 (70), 192 (28), 176 (100), 164 (30), 148 (38), 133 (93), 118 (38). UV spectrum, λ_{max}, nm (log ε): 280 nm (4.19).

SUMMARY

1. A method for obtaining 3-phenacylbenzoxazolinones and 2-(phenacylthio)-benzoxazoles based on the reaction of benzoxazolinone and benzoxazolinethione salts with α-halo carbonyl compounds has been developed.

2. It has been established that the reaction takes place at the less electronegative atom of the ambident benzoxazolinone and benzoxazolinethinone ions.

3. When 3-phenacylbenzoxazolinone is nitrated with nitric acid, the reaction takes place in the benzoxazolinone moiety of the molecule.

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ISOLATION OF A PROTEIN WITH DIAPHORASE ACTIVITY FROM COTTON SEEDS AND A STUDY OF SOME OF ITS PROPERTIES

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The diaphorase activities of the proteins of different varieties of two species of cotton plant — *Gossypium hirsutum* L. and *G. barbadense* L. — have been studied. It has been shown that the proteins of the seeds of the cotton plant *G. barbadense* possess a low diaphorase activity in comparison with the proteins of the seeds of a *G. hirsutum* plant. On an electrophoretogram of the proteins, diaphorase activity was localized in two zones, with R_f 0.45 and 0.70. The diaphorase with R_f 0.45 has been isolated by electrophoresis in polyacrylamide gel (PAAG) and some of its properties have been studied. The diaphorase isolated oxidizes NADH and NADPH in the presence of various artificial electron Acceptors, and has two pH optima (at 7.20 and 8.70) and is characterized by relative thermal stability (at 80°C). In the case of the total extract, brief boiling does not lead to inactivation of the enzyme, which shows the presence in cotton seeds of a factor stabilizing this diaphorase. The molecular weight of the proteins isolated, according to gel filtration on Sephadex G-150, is 59,000, and from the results of SDS-PAAG electrophoresis it is 13,600, which shows a tetrameric structure of the enzyme.

It has been established previously that the seeds of cotton plants of the species *Gossypium hirsutum* L. have a higher level of diaphorase activity than the seeds of the species *G. barbadense* L. [1]. An electrophoretic investigation showed that diaphorase activity was possessed by electrophoretic bands with proteins having mobilities of 0.45 and 0.70, the band with a mobility of 0.45 oxidizing both NADH and NADPH in the presence of artificial electron acceptors [2]. In the present paper we consider the results of a further investigation of difference in the diaphorase activity of these two types of cotton plants and also a study of some properties of the protein isolated.

Diaphorase activity had previously been investigated for five varieties of *G. hirsutum*: S-427, 108-F, Tashkent-1, Tashkent-6, and Mexicanum, and four varieties of *G. barbadense*: S-6030, 8763-1, 9696-1, and 5904-1, at 20°C [1]. In the present stage, we have investigated the diaphorase activity of varieties 153-F, Tashkent-1, AN-chillyaki, AN-402, and Krasnolistnaya akala of the species *G. hirsutum* and S-6030, 5904-I, and 8704-I of the species *G. barbadense*, and this under standard conditions at 30°C.

The results obtained on the NAD(P)H—2,4-dichlorophenolindophenol (DCPIP) oxidoreductase activity of the soluble fraction of the proteins indicated that the level of diaphorase activity in the seeds of the cotton plant *G. hirsutum* was actually higher than in the seeds of *G. barbadense* (μmole/min per mg × 10^2) (see following page)

When these figures compared with those obtained previously [1], it is found that the interspecies difference in diaphorase activities is retained although, formerly [1], considerably lower results had been obtained, apparently because of the temperature factor.

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