PHOSPHORYLATED HYDRAZINIUM SALTS AND THEIR NEUROTROPIC ACTIVITY

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Diphenylphosphinyl acetohydrazide (I), which possesses neurotropic activity, has been recommended for the treatment of a number of nervous conditions [1]. The molecule of I contains the hydrazide moiety, and it may therefore be assumed to dissociate in solution, however, examination of the acid–base equilibrium of the drug showed it to be present in the molecular form over the pH range 3.0-10.0 [4].

In order to study the neurotropic activity of I in the dissociated state, the reaction of the drug with acids has been studied. This results in conversion into the cation and the formation of the [(diphenylphosphinyl)acetyl]hydrazinium salts (IIIa-c). Also prepared were the hydrazinium salts of the phosphorus containing acids IVa and IVb.

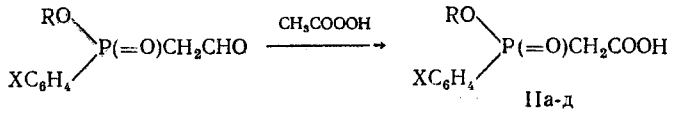
$$(C_6H_5)_2P(=O)CH_2CONHNH_2 + HX \longrightarrow [(C_6H_5)_2P(=O)CH_2CONHNH_3)]^+X^-$$

IIIa: $X = (CH_2=CHCH_2)_2P(=O)O^-$; IIIb: $X = CCl_3COO^-$; IIIc: $X = CF_3COO^-$

$$R^1R^2p(=O)YH + NH_2NH_2 \longrightarrow [NH_2NH_3]^+ [YP(=O)R^1R^2]^-;$$

IVa: $R^1 = R^2 = CH_2=CHCH_2$, $Y = O$; IVb: $R^1 = ClC_6H_4$, $R^2 = OC_2H_5$, $Y = CH_2COO$.

The phosphinic acids required for the preparation of these salts (IIa-e) (Table 1) were obtained by oxidation of the phosphorylated aldehydes with peracetic acid [3]. Under these conditions, selective oxidation of the aldehyde grouping only takes place, the alkoxy-group attached to phosphorus remaining unchanged.



IIa-д

IIa: $X = Cl\text{-}p$, $R = CH_3$; IIb: $X = Cl\text{-}p$, $R = C_2H_5$; IIc: $X = Cl\text{-}p$, $R = C_3H_7\text{-}i$; IId: $X = F\text{-}p$, $R = C_2H_5$; IIe: $X = F\text{-}p$, $R = C_3H_7\text{-}i$.

The IR and PMR spectra of the acids show absorption and signals in accordance with their structural features. The phosphorylated hydrazinium salts (Table 2) were obtained as colorless, crystalline solids which were soluble in water. It was found that in aqueous solution they existed in the dissociated state, the equilibria being shifted towards the formation of ions. For example, the dissociation constant λ_o for the salt IIb was 159.3, and its association constant K_{ass}31*.

The IR spectra of the salts show characteristic multiplet absorption in the 1800-3350 cm^{-1} region [5]. In the IR spectra of salts obtained from I, there are some changes in the positions of the absorption bands as compared with the starting material [2]. In these spectra, there is a broad band with several maxima over the range 2150-2700 cm^{-1}, assigned

*λ_o and K_{ass} were found by I. I. Evgen'ev, whom the authors wish to thank, using the method of Brel and Kraus.

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TABLE 1. Phosphorylated Acetic Acids

Compound	Yield, %	mp, °C	Found, %		Empirical formula	Calculated, %	
			halogen	P		halogen	P
IIa	30	Масло	13,90 13,75	12,52 12,62	$C_9H_{10}ClO_4P$	14,2	12,47
IIb	70	88—89	13,14 13,37	11,67 11,46	$C_{10}H_{12}ClO_4P$	13,14	11,8
IIc	50	92—94	12,41 12,53	11,37 11,33	$C_{11}H_{14}ClO_4P$	12,8	11,21
IId	30	85—86	7,32 7,45	12,50 12,60	$C_{10}H_{12}FO_4P$	7,7	12,6
IIe	30	80—81	6,95 6,71	11,80 12,50	$C_{11}H_{14}FO_4P$	7,3	12,00

TABLE 2. Phosphorylated Hydrazinium Salts

Compound	Yield, %	mp, °C	Found, %		Empirical formula	Calculated, %	
			N	P		N	P
IIIa	60	97—98	6,75 7,04	14,27 14,44	$C_{20}H_{26}N_2O_4P_2$	6,6	14,7
IIIb	50	115	6,90 6,83	6,90 6,81	$C_{16}H_{16}Cl_3N_2O_4P$	6,4	7,09
IIIc	50	129—131	7,04	8,00	$C_{16}H_{16}F_3N_2O_4P$	7,2	7,99
IVa	40	110—113	16,02 16,03	16,85 17,00	$C_3H_{15}N_2O_2P$	15,7	17,4
IVb	60	114—115	9,88 9,87	10,30 10,60	$C_{10}H_{16}ClN_2O_4P$	9,5	10,6

to the N^+H_3 cation. Carbonyl absorption in the cation is seen at 1710 cm^{-1}, i.e., it is shifted towards higher frequencies by 20 cm^{-1} as compared with (I) (ν_{CO} 1690 cm^{-1}). Changes were also observed in the NH absorption. In salts, this corresponds to bands at 3280-3300 cm^{-1}. These results suggest that the positive charge may be distributed over the hydrazide moiety in these salts.

The biological activity of the phosphorylated hydrazinium salts in comparison with that of I was examined using [(diphenylphosphinyl)acetyl]hydrazinium diallylphosphinate (IIIa) as an example.

EXPERIMENTAL (CHEMICAL)

IR spectra were obtained on a UR-20 instrument (East Germany), in vaseline oil. The yields and properties of the compounds obtained are shown in Tables 1 and 2.

p-Fluorophenylethoxyphosphinylacetic Acid (IId). To 9.2 g (0.04 mole) of p-fluorophenylethoxyphosphonylacetaldehyde was added dropwise at 10°C 5.5 g (0.04 mole) of 60% peracetic acid. The mixture was brought to room temperature, and heated for 5 h at 50°C. Acetic acid was removed under reduced pressure, to give an oil which crystallized on standing, The crystals were washed with ether, and dried *in vacuo* to give 3.2 g of IId. Compounds IIa, c, e were obtained similarly.

[(Diphenylphosphinyl)acetyl]hydrazinium Diallylphosphinate (IIIa). I (1.8 g; 0.007 mole) was mixed with 1.46 g (0.01 mole) of diallylphosphinic acid and 3 ml of ethanol, whereupon the temperature rose by 5°C. The solid which separated was filtered off, washed with ether, and dried *in vacuo* to give 1.7 g of IIIa. Compounds IIIb, c were obtained similarly.

Hydrazinium Diallylphosphinate (IVa). Diallylphosphinic acid (3 g; 0.02 mole) was mixed with 1 g (0.04 mole) of hydrazine hydrate. The mixture was kept for 1 h at room temperature, and excess hydrazine hydrate removed under reduced pressure, whereupon the compounds crystallized. The crystals were washed with ether and dried under reduced pressure to give 1.44 g of IVa. IVb was obtained similarly.

EXPERIMENTAL (BIOLOGICAL)

The acute toxicities of (I) and (IIIa) were determined in mice by the intraperitoneal route. The results were evaluated by the Miller and Teitner method. The LD_{50} I was 315 ± 24.8 (262-358) mg/kg, LD_{100} 400 mg/kg, and the maximum tolerated dose 200 mg/kg. For (IIIa), the LD_{50} was 620 ± 23.9 (598.9-671.1) mg/kg, LD_{100} 700 mg/kg, and maximum tolerated dose 500mg/kg.

The symptoms of toxicity both for I and IIIa were depression, with onset lateral posture was not observed. Compound I suppressed the orientation reaction in mice following intraperitoneal administration of doses of 40 mg/kg (1/5 of the MTD) and 25 mg/kg (1/8 of the MTD), $P = 0.001$. IIIa suppressed the orientation reaction when administered by the same route in doses of 62.5 mg/kg (1/8 of the MTD), $P = 0.004$, and 100 mg/kg (1/5 of the MTD), $P = 0.008$. The effects of the compounds on the central-reactive system were examined in mice using a dose of 15 mg/kg intraperitoneally 90 min following treatment with the drug, and arecolinefin a subcutaneous dose of 25 mg/kg 60 min following treatment. I was administered intraperitoneally ina dose of 200 mg/kg, and IIIa in a dose of 300 mg/kg.

It was found that I has central H-cholinolytic effects, since it prevented or reduced nicotine convulsions in mice, and prevented death in 50% of the animals ($P = 0.025$-0.011). Compound IIIa showed only a tendency towards H-cholinolytic activity, since it significantly increased the latent period (time from the administration of nicotine following treatment with the drug to the onset of convulsions) by a factor of 6.7 ($P < 0.001$). Tests using arecoline showed that I has no M-cholinolytic effects, since it did not prevent arecoline tremor. IIIa had an M-cholinomimetic effect, significantly increasing the duration of arecoline tremor by a factor of 3.3 ($P < 0.001$).

The central adrenolytic effects of I and IIIa were assessed by observing the effects of the drugs on the toxicity of amphetamine in grouped mice. I was administered in a dose of 200 mg/kg 80 min before the amphetamine (20 mg/kg subcutaneously), and IIIa in an intraperitoneal dose of 300 mg/kg. Both drugs displayed protectant activity against the group toxicity of amphetamine. I prevented the deaths of 60% of the animals ($P \leq 0.01$), and IIIa of 70% of the animals ($P \leq 0.01$). This is evidence of the central adrenolytic activity of both drugs. In studies of the interaction with convulsant agents, the drugs were administered intraperitoneally 30 min before application of the convulsants, (I) in a dose of 200 mg/kg and IIIa in a dose of 300 mg/kg. The introduction of caffeine–sodium benzoate in a dose of 200 mg/kg subcutaneously following treatment with (I) resulted in powerful convulsions and the deaths of all the experimental animals, whereas in the controls the convulsant effects of caffeine were scarcely apparent. Similar effects were seen on subcutaneous administration of 800 mg/kg of caffeine following treatment with IIIa. Treatment with corazole in a subcutaneous dose of 125 mg/kg following treatment with either drug resulted in increased severity of convulsions and an increase in their duration in comparison with the controls. The drugs failed to prevent the deaths of the mice. Subcutaneous administration of strychnine in a dose of 1 mg/kg following treatment with (I) resulted in convulsions and death in both the experimental and the control mice, but the mice in the experimental group showed increased lifespan by a factor of four ($P = 0.01$). When the dose of strychnine was 2 mg/kg, prior treatment with IIIa resulted in an 8.4-fold increase in the lifespan ($P = 0.006$).

Hence, neither drug shows anticonvulsant activity, but rather they increase the sensitivity of the mice to corazole and caffeine. In studying the interactions of the drugs with narcotics and soporofics, tests were carried out using hexobarbital and barbital sodium. The onset of sleep was taken as the time at which the lateral posture was adopted. Joint treatment with I and hexobarbital showed that the latter, when administered intraperitoneally in a dose of 100 mg/kg 60 minutes after intraperitoneal administration of I in a dose of 100 mg/kg ($\frac{1}{2}$ MTD), caused a sixfold increase in the duration of sleep as compared with the controls ($P < 0.001$). Further treatment with I in the same dose immediately following the awakening of the mice again caused them to fall asleep, the duration of sleep being three times longer than the initial hexobarbital sleep in the control mice ($P < 0.001$). Combined treatment with IIIa (1/5 MTD, 100 mg/kg intraperitoneally) and hexobarbital (60 mg/kg intraperitoneally) showed that this drug increased the duration of hexobarbital sleep by a factor of 5.6 ($P < 0.001$). Administration of the drug immediately following awakening did not result in further sleep. Administration of barbital sodium (175 mg/kg intraperitoneally) 90 min following treatment with I (200 mg/kg intraperitoneally) resulted in duration of sleep 2.5

times greater than in the controls (P = 0.05). When (I) was administered to the mice in the same dose following awakening from the barbital-sodium-induced sleep, the animals again fell asleep, the duration of sleep being 1.6 times greater than the original barbital-sodium-induced sleep (P = 0.05). Intraperitoneal administration of IIIa in a dose of 100 mg/kg (1/5 of the MTD) 90 min before barbital sodium (175 mg/kg intraperitoneally) did not potentiate sleep in mice. This provided confirmation that this drug does not behave as a "true" potentiator of soporofic and narcotic drugs.

Studies of analgesic activity showed that both drugs possess this activity in doses of from $\frac{1}{2}$ of the MTD upwards. For (I), this was 100 mg/kg, and for IIIa, 250 mg/kg. At the maximum tolerated doses, the analgesic effects of both I and IIIa lasted for more than five hours.

Comparing the results obtained with IIIa with those obtained for I, it will be seen that IIIa is only half as toxic as I, and that suppression of the orientational reaction is seen at the same dose as for I. Both IIIa and I enhance to the same extent the effects of such convulsants as caffeine and corazole.

Comparison of the effects of I and IIIa on the central choline-reactive systems shows that IIIa displays central M-cholinomimetic activity which is not shown by I. I has high central H-cholinolytic activity, whereas IIIa exhibits only a tendency to H-cholinolytic activity in the shape of an increase in the duration of the latent period before the onset of nicotine convulsions. The drugs have the same central adrenolytic activity. I is a "true" potentiator of narcotic and soporific effects, whereas IIIa does not display this activity.

The compounds are equally active as analgesics. Thus, in its depressant effects on the central nervous system IIIa differs from I, but possesses no advantages over it. However, the high biological activity of IIIa encourages further studies of modifications of (I).

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