Heterocycles Structurally Influenced by a Side Chain. II.[1] 7-Phenacylxanthopterins and 6-Phenacylisoxanthopterins[2]

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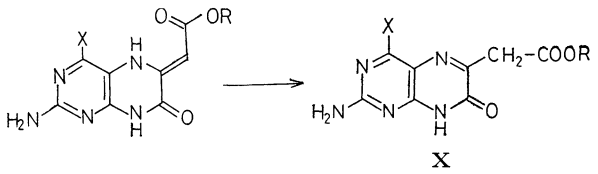
Phenacyl derivatives of two naturally-occurring pteridines and their 4-amino analogues (III and V) have been synthesized for their structural study. Since these compounds are obtained by the condensation of ethyl benzoylpyruvates (II) with tri- or tetraaminopyrimidine (I), a carbon-nitrogen double bond, which is adjacent to the phenyl group of the side chain, may be expected in the ring (–N=Ċ–CH_2–CO–Ph). However, their NMR spectra show that the double bonds of all the products are displaced onto the side chain (–NH–Ċ=CH–CO–Ph), the carbonyl of which is hydrogen-bonded with the secondary amino group thereby formed. This form of V makes striking contrast to alkyl isoxanthopterin-6-acetates (X), in which the corresponding double bond remains undisplaced. The intermediate products of V (IV) and V were hydrolyzed with acid or alkali into 6-methylisoxanthopterin (VI) and benzoic acids (VII).

When a side chain with a β-carbonyl group is introduced into nitrogen heterocycles at a position adjacent to the nitrogen atom, the carbon-nitrogen double bond of the ring is often displaced onto the side chain.[1] This displacement is likely to be facilitated by an intramolecular hydrogen bonding between the carbonyl and the secondary amino group of the ring thereby formed. One of our early reports[3] was the first presenting evidence for the fixation of the enamine form, which is illustrated below:

Imine form Enamine form

It is said that the imine form rather than the enamine form is predominant in imine-enamine tautomerism when hydrogen is present on the nitrogen atom.[4,5] However, the enamines reported[6] on by our laboratory are stable under ordinary conditions in spite of the presence of hydrogen.

During the course of further studies, many examples of similarly-fixed enamines in condensed[1,3,6,7] and noncondensed[3,6,7] heterocycles of five-,[7] six-,[1,3,6,7] and seven-membered[7] rings, and also in simpler alkyl β-aminoacrylates,[8] have been encountered. Interestingly, however, a structural resemblance is not always followed by such a fixation of the enamine form. Indeed, reverse is the case in alkyl isoxanthopterin-6-acetates and their 4-amino analogues (X), which exist in the imine form, as we recently reported.[9] It appears that the imine forms of these compounds are also fixed unless otherwise dissolved in a protic solvent.[9]



X

X=O or NH
R=CH_3 or C_2H_5

Consequently, we were particularly interested in whether or not the phenacyl derivatives of xanthopterin and isoxanthopterin exist in the enamine forms. We will now describe the synthesis and characterization of the phenacyl derivatives of these two naturally-occurring pteridines (originally found in butterfly wings), all of which are new compounds.

The condensation of 2,5,6-triamino-4-hydroxypyrimidine (Ia) with ethyl p-substituted benzoylpyruvate (or the corresponding acid in some cases) (II) under acidic conditions gave 7-(p-substituted phenacyl)xanthopterin (III), but in an ammoniacal solution it afforded an intermediate product (IV), which was then cyclized to 6-(p-substituted phenacyl)isoxanthopterin (V) in the presence of sulfuric acid. The 4-amino analogues of these three types of products (III, IV, V; X=NH_2) could be obtained by the reaction of 2,4,5,6-tetraaminopyrimidine (Ib) instead of Ia. The position of the phenacyl groups in V was confirmed by the fact that V was hydrolyzed into 6-methylisoxanthopterin (VI) and the corresponding benzoic acid (VII). A similar hydrolysis proceeded with the intermediate product (IV).

All these phenacylpteridines (III and V) were sparingly soluble or practically insoluble in the usual solvents, but could be dissolved into concentrated sulfuric acid. Thus, the NMR spectra of these com-

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1) Part I: Y. Iwanami, T. Seki, and T. Inagaki, This Bulletin, **44**, 1316 (1971).

2) Presented in part at the IVth International Symposium on Pteridines, Toba, July, 1969, and also partially described in the Symposium Proceedings, "Chemistry and Biology of Pteridines," distributed by Maruzen Co., Tokyo (1970), p. 129.

3) Y. Iwanami, *Nippon Kagaku Zasshi*, **82**, 778, 780 (1961).

4) J. March, "Advanced Organic Chemistry: Reactions, Mechanisms, and Structure," McGraw-Hill Book Co., New York (1968), p. 62.

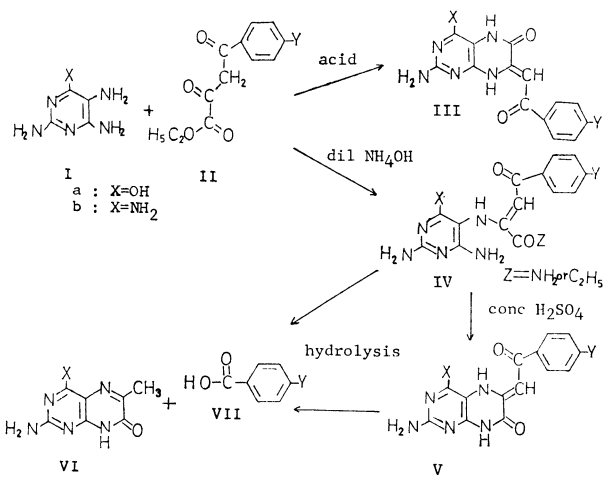
5) J. D. Roberts and M. C. Caserio, "Basic Principles of Organic Chemistry," W. A. Benjamin, New York (1964), p. 486.

6) Y. Iwanami, This Bulletin, **44**, 1311 (1971); Y. Iwanami, Y. Kenjo, K. Nishibe, M. Kajiura, and S. Isoyama, *ibid.*, **37**, 1740 (1964); Y. Iwanami, S. Isoyama, and Y. Kenjo, *ibid.*, **37**, 1745 (1964)

7) Y. Iwanami, *Nippon Kagaku Zasshi*, **83**, 100, 161, 316, 593, 597 (1962); H. Sasaki, H. Sakata, and Y. Iwanami, *ibid.*, **85**, 704 (1964).

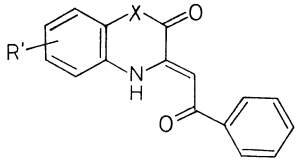
8) Y. Iwanami, *ibid.*, **82**, 632, 634 (1961); **83**, 600 (1962).

9) Y. Iwanami, This Bulletin, **44**, 1314 (1971).



pounds were determined in sulfuric and deuteriosulfuric acid. 7-(*p*-Methylphenacyl)xanthopterin (IIIb), for example, exhibits no signal between 7.19 and 0 ppm (δ scale) except for a singlet at 2.60 ppm due to the methyl group which is attached to the benzene ring of IIIb. Therefore, IIIb does not involve methylene ($-CH_2-$) in its structure. Instead, it contains a methine (=CH–) group, which is revealed by a singlet at 7.19 ppm. The bands in the lowest field, *ca.* 8.3 ppm, must be due to the protons *ortho* to the electron-withdrawing carbonyl group. This, in turn, indicates that the carbonyl group does not exist as an enol in IIIb. Since the enol double bond is thus absent, the above methine can be assigned to another double bond, which is derived by the migration of the carbon-nitrogen double bond of the ring onto the side chain.

The NMR data obtained on the samples of the other analogues, III and V, are similar with those of this one, IIIb. It is, therefore, fitting to call them by the more practical expression "7-(*p*-methylphenecylidene)-7,8-dihydroxanthopterin" (IIIb) and so on. This type of partial structure of III and V is exactly the same as that of 3-phenacyl-2(1*H*)-quinoxalinones (VIII, X=NH) and 3-phenacyl-2*H*-1,4-benzoxazin-2-ones (VIII, X=O), which have been shown to exist in the "phenacylidene" structure (*i.e.*, the enamine form) both in solution and in crystalline states, as reported in a preceding paper.[1]

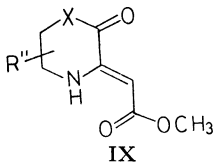


VIII

X=O or NH
R′=H, CH_3, Cl, or Benzo

In the case of VIII, an amino proton gave rise to a band in the lowest field (13.88—12.02 ppm), giving evidence of its participation in the intramolecular hydrogen bonding. In the present study, however, we could not observe the corresponding signal. The signal of the amino proton is probably overlapped with that of the solvent (11.38 ppm). Moreover, sulfuric acid is not a proper solvent for the NMR spectra of the intermediate (IV). Although IV exhibited an olefinic proton signal, this might be due to the methine of V, which could be derived from dehydration with sulfuric acid. Finally, we found that dimethyl sulfoxide-d_6 dissolved IV as well as III and V to a certain extent at a high temperature (175°C). In this solvent, the products, IIIb, IVb, and Vb, for example, gave methine proton signals at 6.54, 5.88, and 5.90 ppm respectively, in addition to the aromatic *ortho* protons around 8 ppm. This also supports the enamine structures of these three types of products.[10,11]

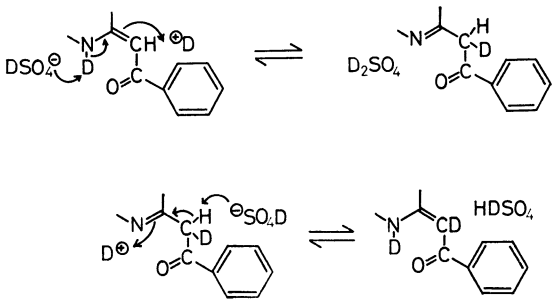
Returning to the NMR spectra in the sulfuric-acid solvent system, the profile of the spectrum of IIIb in sulfuric acid-d_2 was not the same as that in sulfuric acid. The methine proton signal disappeared in sulfuric acid-d_2. This accounts for the deuterium exchange. The exchange mechanism would be a concerted reaction,[9] similar to that proposed for VIII in trifluoroacetic acid-d[1] and to that for the compounds with the general formula of IX,[6] as is illustrated below:



IX

X=O or NH
R″=Benzo or H

In trifluoroacetic acid, VIII exists in an equilibrium mixture of four forms, including not only the imine-enamine tautomers but also the keto-enol tautomers of a phenacyl moiety.[1] However, III, IV, and V, all possessing the same phenacyl group, gave no spectral indications of the keto-enol tautomers. There was thus a most impressive contrast between the products of this study and VIII, in spite of their structural resemblance. Therefore, the deuterium exchange mechanism of III, IV, and V is more closely related to that of IX,[6] which does not have a phenacyl substituent, and they must be essentially the same:



One of the possible exchange mechanisms

There is another striking contrast between V and alkyl isoxanthopterin-6-acetates (X).[9] Both of these compounds are 6-substituted derivatives of isoxan-

10) Since the spectra of VIII in this solvent are essentially the same (the enamine type) between 35 and 175°C, and since the spectra of X are also the same (the imine type), in this temperature range, we assume that the present products exist in the enamine form not only at 170° but also at lower temperatures.

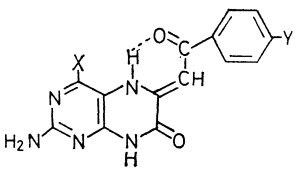
11) The IR spectra of III, IV, and V are not so informative because of their complicated bands.

TABLE 1. ELECTRONIC ABSORPTION MAXIMA OF THE PRODUCTS

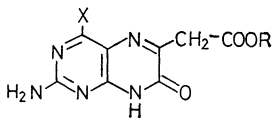
Compound		Substituents X	Y	Z	λ_{max} nm ($\times 10^{-3}$) in 0.1N NaOH			
7-(*p*-Substitued phenacylidene)-7,8-dihydroxanthopterins	IIIa	OH	H		228 (34.5)		345 (11.0)	422 (11.5)
	IIIb	OH	CH_3		227 (31.9)	258 (18.8)	340 (9.4)	418 (14.7)
	IIIc	OH	Cl		225 (37.2)		335 (8.4)	433 (23.4)
	IIId	OH	Br		228 (33.9)		335 (8.8)	433 (20.7)
	IIIf	OH	CN		233 (34.6)			450 (21.1)
	IIIg	OH	COOH		231 (26.3)		340 (12.7)	446 (15.4)
	IIIa′	NH_2	H		242 (17.5)		325 (8.5)	470 (20.1)
	IIIb′	NH_2	CH_3		223 (37.0)	258 (26.0)	343 (15.1)	410 (3.2)
	IIIe′	NH_2	OH		223 (40.7)		339 (35.3)	
	IIIf′	NH_2	CN		227 (36.9)		343 (10.2)	441 (18.9)
2,6-Diamino-4-hydroxy-(or amino) pyrimid-5-ylamino (*p*-substituted phenacylidene) acetates	IVa	OH	H	NH_2	240 (23.1)		356 (9.8)	
	IVb	OH	CH_3	NH_2	251 (25.6)		360 (10.0)	
	IVe	OH	OH	NH_2	225 (32.0)		340 (23.6)	
	IVg′	OH	$CONH_2$	OH	232 (23.5)		350 (11.3)	440^{sh} (8.5)
	IVe′	NH_2	OH	OC_2H_5	223 (40.3)		339 (37.2)	
6-(*p*-Substituted phenacylidene)-5,6-dihydroisoxanthopterins	Va	OH	H			260 (——)	377 (——)	
	Vb	OH	CH_3			258 (20.8)	408 (17.5)	
	Vc	OH	Cl			252 (——)	374 (——)	
	Vd	OH	Br			271 (22.1)	377 (12.0)	
	Ve	OH	OH		243 (19.8)	283 (13.9)	322 (20.0)	440 (43.9)
	Vg	OH	COOH		233 (28.2)		343 (14.2)	438 (14.9)

Marks (——): Values undeterminable because of very limited solubility.
sh: Shoulder. All these compounds are sparingly soluble in dilute acids, and show melting points above 300°C.

thopterin, but they are different from each other in their existing forms; *i.e.*, V exists in the enamine form, and X in the imine form.



V



X

The replacement of an alkoxyl group in X by a phenyl group into V makes an extension of the conjugated system in the enamine form to include the benzene ring, so that the form is stabilized.[12]

None of the products, III, IV, or V, melt below 300°C. The maxima of the electronic absorption spectra of these products in 0.1N sodium hydroxide are listed in Table 1.

The NMR spectra show that the present products exist in the enamine form. The signals of the corresponding imine form do not appear in our results. The only indication of its existence was given when the spectra were measured in sulfuric acid-d_2. In other words, the fact that the methine proton signal disappeared upon deuterium exchange was interpreted to indicate the presence of the imine form[14] in a trace amount. The previous observations on VIII and IX indicated that the methine proton siganl of VIII in dimethyl sulfoxide-d_6 did not disappear when deuterium oxide was added (*i.e.*, exclusively in the enamine form), and that of IX in methanol-d_4 also remained unaffected; besides, the enamine structures of VIII and IX were fixed even in the crystalline state, as was proved by the IR spectroscopy. In a protic solvent like trifluoroacetic acid, however, VIII and IX have been shown to exist as an equilibrium mixture of imine and enamine tautomers,[15] because both methylene (imine) and methine (enamine) proton signals were observed, and were removed in trifluoroacetic acid-*d*. On the bases of these observations, we deduce that the present products, III, IV, and V, are fixed in the enamine structure unless dissolved in a protic solvent like sulfuric acid.

12) This is probably not the only factor in inducing the enamine form. The influence of phenyl substitution in the present comparison, however, would be similar to that of benzoylacetone (89.2% enolized) in contrast to ethyl acetoacetate (8.0% enolized).[13]

13) J. March, "Advanced Organic Chemistry: Reactions, Mechanisms, and Structure," McGraw-Hill Book Co., New York (1968), p. 60.

14) The other interpretation would be a similar migration and return of the double bond *via* its immonium ion, as was illustrated in a previous paper,[9] (this mechanism is at present indistinguishable from the concerted reaction already mentioned) or the direct ionization of the methine hydrogen (carbanion formation).

15) Many examples of NMR spectra which separately indicate both forms of keto-enol tautomers, and of imine-enamine tautomers[1,6,16] as well, are known. This suggests that, when either one of the forms is shown solely in the spectrum, the other form is improbable or exists in a very small amount, beneath the lebel of detection, rather than that the rapid interconversion (indistinguishable by a NMR spectrometer) of two (or more) forms gives rise to a spectrum of an appearently single form. In the latter case, it is most probable that all the signals of protons taking part in the interconversion fall into a single peak, together with that of solvent acid, at a moderate temperature, because hydroxyl or amino proton exchanging with the solvent proton rapidly constitutes one signal joined together.

16) L. Merlini, W. von Philipsborn, and M. Viscontini, *Helv. Chim. Acta*, **46**, 2592 (1963).

Even in this acid, the compounds mostly exist in the enamine form, as has already been mentioned.

Besides the physicochemical observations, we made a study of the biological effects of the products, III, IV, and V. Of these compounds, some of the IV compounds were shown to stimulate the cell growth of several microorganisms. The most active substance was 2,6-diamino-4-hydroxypyrimid-5-ylamino(*p*-hydroxyphenacylidene)acetamide (IVe). This compound remarkedly enriched the cell mass of *Escherichia coli*, *Clostridium butyricum*, *Candida utilis*, and *Rhodotorula glutinis* to two- or three-fold that of the control cultures.[17]

Experimental

The NMR spectra were determined on a Hitachi H-60 spectrometer, while the electronic absorption were taken on a Hitachi 124 spectrophotometer. All the melting points are uncorrected.

Ethyl (p-Substituted Benzoyl)pyruvates (IId, g, g').[18-20] Ethyl *p*-bromobenzoylpyruvate (IId) (yield 68%); colorless needles; mp 60.5—61.5°C.

Found: C, 48.50; H, 3.86%. Calcd for $C_{12}H_{11}O_4Br$: C, 48.19; H, 3.63%.

Ethyl *p*-carboxybenzoylpyruvate (IIg) (53%); colorless needles; mp 126—128°C.

Found: C, 58.97; H, 4.36%. Calcd for $C_{13}H_{12}O_6$: C, 59.09; H, 4.58%.

p-Ethoxycarbonylbenzoylpyruvic acid (IIg'). Ethyl *p*-ethoxycarbonylbenzoylpyruvate, prepared from *p*-ethoxycarbonylacetophenone and diethyl oxalate in a 45% yield, was hydrolyzed with a mixture of 10% sulfuric acid and acetic acid (2:1) to give IIg' as colorless crystals (40%); mp 150—151°C.

Found: C, 55.68; H, 4.85%. Calcd for $C_{13}H_{12}O_6 \cdot H_2O$: C, 55.32; H, 5.00%.

7-Phenacylidene-7,8-dihydroxanthopterin (IIIa).[21] Into a warmed (*ca.* 50°C) suspension of 2,5,6-triamino-4-hydroxypyrimidine (IIa) sulfate (2.4 g) in water (50 m*l*), a solution of ethyl benzoylpyruvate (Ia) sodium salt (3.6 g) in 1N acetic acid (100 m*l*) was dropped with stirring, and then the temperature of the reaction mixture was gradually raised to 100°C. The mixture, which started to turn a deep orange with the formation of reddish precipitates, was refluxed for 1.5 hr, and then left to stand overnight at room temperature with continuous stirring. The crystals thus deposited were collected on a funnel, and washed well with water and ethanol. The crude product (2.4 g) was recrystallized from dimethyl sulfoxide to give IIIa as orange crystals; mp>300°C.

Found: C, 52.83; H, 4.65; N, 22.63%. Calcd for $C_{14}H_{11}O_3N_5 \cdot H_2O$: C, 53.33; H, 4.16; N, 22.22%.

7-(p-Methylphenacylidene)-7,8-dihydroxanthopterin (IIIb).[21] In to a warmed (*ca.* 50°C), a solution of IIb (1.9 g) in ethanol (50 m*l*) was dropped with stirring. The reaction mixture was gradually heated, and then refluxed for 1.5 hr to give an orange precipitate. After the mixture had been left to stand overnight at room temperature with stirring, the deposited crystals were collected on a funnel, and washed with water and ethanol. The crude product (1.4 g) was repeatedly recrystallized from dimethyl sulfoxide to give IIIb as orange-yellow crystals; mp>300°C.

Found: C, 54.47; H, 4.20; N, 21.36%. Calcd for $C_{15}H_{13}O_3N_5 \cdot H_2O$: C, 54.71; H, 4.59; N, 21.27%.

7-(p-Chlorophenacylidene)-7,8-dihydroxanthopterin (IIIc).[21] In a manner similar to that used in the preparation of IIIb, the crude product of IIIc (1.4 g) was obtained from Ia sulfate (1.2 g) and IIc (2.1 g). The product was recrystallized from dimethyl sulfoxide to afford IIIc as yellow prisms; mp>300°C.

Found: C, 50.46; H, 3.12; N, 20.95%. Calcd for $C_{14}H_{10}O_3N_5Cl$: C, 50.71; H, 3.02; N, 21.11%.

7-(p-Bromophenacylidene)-7,8-dihydroxanthopterin (IIId). The crude product of IIId (2.8 g) from Ia sulfate (1.2 g) and IId sodium salt (2.4 g) was recrystallized from dimethyl sulfoxide to give IIId as yellow prisms; mp>300°C.

Found: C, 44.42; H, 2.83; N, 18.33%. Calcd for $C_{14}H_{10}O_3N_5Br$: C, 44.71; H, 2.66; N, 18.61%.

7-(p-Cyanophenacylidene)-7,8-dihydroxanthopterin (IIIf). The crude product of IIIf (0.75 g) from Ia sulfate (0.96 g) and IIf sodium salt (1.2 g) was recrystallized from dimethyl sulfoxide to afford IIIf as brownish red crystals; mp>300°C.

Found: C, 51.10; H, 3.77; N, 23.76%. Calcd for $C_{15}H_{10}O_3N_6 \cdot 3/2H_2O$: C, 51.60; H, 3.72; N, 24.06%.

7-(p-Carboxyphenacylidene)-7,8-dihydroxanthopterin (IIIg). The crude product of IIIg (2.0 g) from Ia sulfate (1.8 g) and IIg (2.0 g) was recrystallized from dimethyl sulfoxide to give IIIg as brownish red crystals; mp>300°C.

Found: C, 48.49; H, 4.05; N, 18.63%. Calcd for $C_{15}H_{11}O_5N_5 \cdot 3/2H_2O$: C, 48.91; H, 3.83; N, 19.02%.

7-Phenacylidene-2,4-diamino-6(5H)-pteridinone (IIIa').[21] Into a suspension of 2,4,5,6-tetraaminopyrimidine (Ib) sulfate (1.4 g), a hot solution of IIa sodium salt (2.4 g) was dropped with stirring. The reaction mixture was then heated to 100°C for 2 hr, and thereafter maintained at pH 1—2. After the mixture had stood overnight, the precipitated crystals were collected by filtration. The crude product, IIIa' (0.8 g), was purified by reprecipitation. The crystalls were dissolved in a minimum amount of concentrated sulfuric acid, and then water was added into the solution chilled in an ice bath to give IIIa' as brownish red crystals; mp> 300°C.

Found: C, 50.65; H, 4.03; N, 24.74%. Calcd for $C_{14}H_{12}O_2N_6 \cdot 1/3H_2SO_4 \cdot 1/3H_2O$: C, 50.19; H, 4.01; N, 25.09%.

7-(p-Methylphenacylidene)-2,4-diamino-6(5H)-pteridinone (IIIb').[21] The crude product of IIIb' (1.4 g) from Ib sulfate (1.4 g) and IIb sodium salt (1.7 g) was recrystallized from dimethyl sulfoxide to give IIIb' as yellow crystals; mp>300°C.

Found: C, 56.41; H, 4.87; N, 26.68%. Calcd for $C_{15}H_{14}O_2N_6 \cdot 1/2H_2O$: C, 56.44; H, 4.70; N, 26.32%.

7-(p-Hydroxyphenacylidene)-2,4-diamino-6(5H)-pteridinone (IIIe'). The crude product of IIIe' (1.0 g) from Ib sulfate (1.4 g) and IIe sodium salt (3.2 g) was recrystallized from dimethyl sulfoxide to give IIIe' as orange crystals; mp>300°C.

Found: C, 50.63; H, 4.41; N, 25.42%. Calcd for $C_{14}H_{12}O_3N_6 \cdot H_2O$: C, 50.91; H, 4.27; N, 25.45%.

7-(p-Cyanophenacylidene)-2,4-diamino-6(5H)-pteridinone (IIIf'). The crude product of IIIf' (1.3 g) from Ib sulfate (1.4 g) and IIf sodium salt (1.8 g) was recrystallized from dimethy sulfoxide to give IIIf' as brownish red crystals; mp>300°C.

Found: C, 53.97; H, 4.09; N, 28.86%. Calcd for $C_{15}H_{11}O_2N_7 \cdot 3/4H_2O$: C, 53.81; H, 3.76; N, 29.29%.

2,6-Diamino-4-hydroxypyrimid-5-ylamino(phenacylidene)acet-

17) Details will be published elsewhere.
18) C. Beyer and L. Claisen, *Ber.*, **20**, 2181 (1887).
19) J. B. Wright, U.S. 3223703 (1965).
20) V. A. Zagorevskii and D. A. Zykov, *Zh. Obshch. Khim.*, **33**, 2426 (1963).
21) The mass spectral features of this compound will be reported elsewhere.

amide (IVa). Into a suspension of Ia sulfate (2.0 g) in water (50 ml), 1N ammonium hydroxide (50 ml) was added to alkalize it at about pH 8. A solution of IIa (1.7 g) in ethanol (20 ml) was then dropped with stirring into the suspension, the color of which had turned violet, during a course of about 30 min at room temperature; the pH was maintained by adding 1N ammonium hydroxide. The color of the reaction mixture then became gradually reddish-brown. After the IIa had all been dropped, the mixture was stirred more. In a little while, fine brown crystals began to deposit on the bottom of the reaction vessel. Stirring was continued overnight. The precipitated crystals were collected on a glass filter, and washed successively with 1N sulfuric acid, water, and ethanol. The crude product of IVa sulfate (1.0 g) was recrystallized from dimethy sulfoxide to give yellowish-brown crystals; mp>300°C.

Found: C, 49.66; H, 3.86; N, 24.62%. Calcd for $C_{14}H_{14}O_3N_6 \cdot 1/4H_2SO_4$: C, 49.63; H, 4.31; N, 24.81%.

2,6-Diamino-4-hydroxypyrimid-5-ylamino(p-methylphenacylidene)-acetamide (IVb). In a manner similar to that used in the preparation of IVa, the crude product of IVb (0.9 g) was obtained from Ia sulfate (2.0 g) and IIb (1.8 g). The IVb sulfate was recrystallized from dimethyl sulfoxide to afford IVb as dark yellow crystals; mp>300°C.

Found: C, 52.02; H, 4.47; N, 24.06%. Calcd for $C_{15}H_{16}O_3N_6 \cdot 1/5H_2SO_4$: C, 51.78; H, 4.75; N, 24.16%.

2,6-Diamino-4-hydroxypyrimid-5-ylamino(p-hydroxyphenacylidene)-acetamide (IVe). The crude product (1.6 g) from Ia sulfate (2.4 g) and IIe (2.4 g) was recrystallized from dimethyl sulfoxide to afford IVe as reddish-brown crystals; mp>300°C.

Found: C, 47.22; H, 4.13; N, 23.67%. Calcd for $C_{14}H_{14}O_4N_6 \cdot 3/2H_2O$: C, 47.05; H, 4.79; N, 23.52%.

2,6-Diamino-4-hydroxypyrimid-5-ylamino(p-carbamoylphenacylidene)acetic Acid (IVg'). The crude product (1.4 g) from Ia sulfate (1.8 g) and IIg (2.0 g) was dissolved in 1N sodium hydroxide, and the resulting solution was poured into warm 3N hydrochloric acid. The reprecipitation was repeated three times to give IVg' as dark red crystals; mp>300°C.

Found: C, 46.87; H, 3.93; N, 21.45%. Calcd for $C_{15}H_{12}O_5N_6 \cdot 3/2H_2O$: C, 47.00; H, 3.94; N, 21.93%.

Ethyl 2,4,6-Triaminopyrimid-5-ylamino(phenacylidene) acetate (IVe'). The crude product (0.3 g) obtained by the condensation of Ib sulfate (2.4 g) with IIa (2.2 g) in an ammoniacal solution of pH 7.7 was washed successively with a 1:1 mixture of dimethyl sulfoxide and 50% sulfuric acid, water, and ethanol on a glass filter, and was then recrystallized from dimethyl sulfoxide to give IVe' as reddish-brown crystals; mp>300°C.

Found: C, 45.92; H, 4.42; N, 20.05%. Calcd for $C_{16}H_{18}O_4N_6 \cdot 1/2H_2SO_4 \cdot 1/2H_2O$: C, 46.16; H, 4.84; N, 20.18%.

6-Phenacylidene-5,6-dihydroisoxanthopterin (Va).[21] The The crude product of IVa (1.0 g) was dissolved in a minimum amount of concentrated sulfuric acid. The dark-colored solution was gradually heated to 60°C. After it had been left to stand overnight at room temperature, water was added to the solution chilled in an ice bath; this step was then repeated to give Va(0.5 g) as yellowish-brown crystals; mp> 300°C.

Found: C, 46.46; H, 3.82; N, 18.89%. Calcd for $C_{14}H_{11}O_3N_5 \cdot 1/2H_2SO_4 \cdot H_2O$: C, 46.15; H, 3.87; N, 19.22%.

6-(p-Methylphenacylidene)-5,6-dihydroisoxanthopterin (Vb).[21] In a similar manner, the product, IVb (0.8 g), obtained from Ia sulfate (2.0 g) and IIb (1.8 g) was cyclized in the presence of concentrated sulfuric acid, and was then recrystallized from dimethyl sulfoxide to give Vb as dark orange crystals; mp>300°C.

Found: C, 54.89; H, 4.17; N, 21.15%. Calcd for $C_{15}H_{13}O_3N_5 \cdot H_2O$: C, 54.71; H, 4.59; N, 21.27%.

6-(p-Chlorophenacylidene)-5,6-dihydroisoxanthopterin (Vc).[21] The crude product (1.4 g) from Ia sulfate (2.0 g) and IIc (2.0 g) was recrystallized for hot dimethyl sulfoxide, in which cyclization probably took place, to give Vc as dark yellow crystals; mp>300°C.

Found: C, 50.28; H, 3.23; N, 20.57%. Calcd for $C_{14}H_{10}O_3N_5Cl \cdot 1/4H_2O$: C, 50.04; H, 3.15; N, 20.85%.

6-(p-Bromophenacylidene)-5,6-dihydroisoxanthopterin (Vd). The crude product (1.0 g) form Ia sulfate (2.4 g) and IId (3.0 g) was recrystallized from hot dimethyl sulfoxide to afford Vd as dark yellow crystals; mp>300°C.

Found: C, 44.97; H, 3.03; N, 18.87%. Calcd for $C_{14}H_{10}O_3N_5Br$: C, 44.71; H, 2.66; N, 18.61%.

6-(p-Hydroxyphenacylidene)-5,6-dihydroisoxanthopterin (Ve).[21] The crude product (2.1 g) from Ia sulfate (2.4 g) and IIe (2.3 g) was purified by reprecipitation from a cencentrated sulfuric acid solution to give Ve sulfate as yellow crystals; mp>300°C.

Found: C, 41.25; H, 3.50; N, 16.51%. Calcd for $C_{14}H_{11}O_4N_5 \cdot H_2SO_4$: C, 40.87; H, 3.18; N, 17.03%.

6-(p-Carboxyphenacylidene)-5,6-dihydroisoxanthopterin (Vg). The crude product (0.7 g) from Ia sulfate (0.8 g) and IIg (1.0 g) was reprecipitated from a dimethyl sulfoxide solution by adding water to give Vg as orange-red crystals; mp> 300°C.

Found: C, 48.30; H, 4.23; N, 18.41%. Calcd for $C_{15}H_{11}O_5N_5 \cdot 2H_2O$: C, 47.75; H, 4.01; N, 18.56%.

Hydrolysis of V and IV. A suspension of Ve (0.6 g) 2N sodium hydroxide (30 ml) was refluxed for 30 min, and then left standing to cool. The precipitate thereby formed was removed by filtration. The filtrate was acidified with 6N hydrochloric acid. The yellow crystals thus precipitated were collected on a funnel and washed repeatedly with ethanol. The crystals were dissolved into 1/2N sodium hydroxide, and then 2N hydrochloric acid was dropped into the solution to reprecipitate it. The purification step was repeated three times to afford 6-methylisoxanthopterin (VI) (0.2 g). The UV spectrum was identical with that previously reported.[22] Found: N, 36.69%.

From the combined ethanol extracts, the solvent was removed *in vacuo*; the residue was recrystallized from a mixture of ethanol and ether to give *p*-chlorobenzoic acid (VIIc); mp 243°C, undepressed on admixture with a commercial sample. The identity was also proved by UV spectroscopy.

Found: C, 53.67; H, 3.47%.

A suspension of Vc (0.5 g) in 6N hydrochloric acid (200 ml) was refluxed for 28 hr. The undissolved meterials were removed by filtration while the reaction mixture was still hot. The filtrate was then concentrated under reduced pressure, and the residue was repeatedly extracted with ether. The remaining crystals (0.2 g) were purified according to the above reprecipitation. The identity of the VI thereby formed with the corresponding product in the above alkaline hydrolysis and that of the VIIc obtained from the combined ether extracts with a commercial sample were proved by UV spectroscopy.

A suspension of VIe (0.5 g) in 2N sodium hydroxide (40 ml) was refluxed for 3 hr. After acidification followed by evaporation *in vacuo*, the residue was washed ethanol. The crystals were similarly purified to give VI, which was spectroscopically identical with the sample. The *p*-hydroxybenzoic acid (VIIe) obtained from the combined extracts

22) S. Matsuura, S. Nawa, H. Kakizawa, and Y. Hirata, *J. Amer. Chem. Soc.*, **75**, 4446 (1953).

was identified by a mixed-melting-point determination (mp 213—214°C) and UV spectroscopy.

The other compounds belonging to IV or V (both X=OH) behaved similarly under hydrolysis, as evidenced by the UV spectra of the hydrolysates.

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