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A NOVEL PHOTO-INDUCED METHYLATION OF PYRIMIDINES AND CONDENSED PYRIMIDINES†

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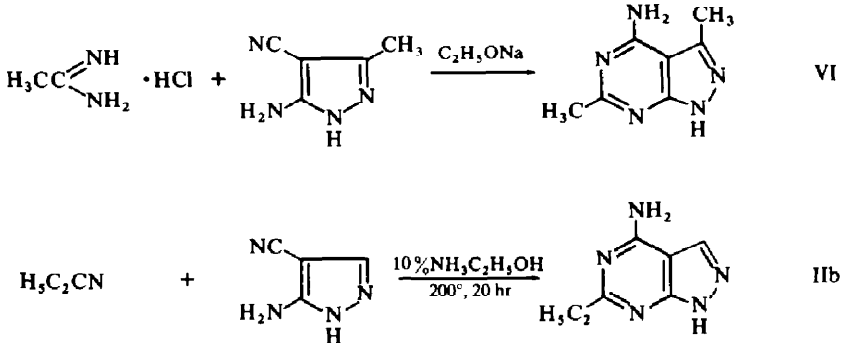
Abstract—A new photo-induced methylation of pyrimidines and condensed pyrimidines by irradiating an acidic solution of the bases in methanol is described. The scope and limitation, effects of acids, the calculation of the reactivity indices by the MO method, effect of bases on $\cdot CH_2OH$ radical formation, and the reaction mechanism are discussed.

THE present paper deals with further aspects of the new photo-induced methylation of pyrimidines and condensed pyrimidines published in a previous communication.[1]

The reaction conditions and the yields of methylation or other alkylations in the pyrimidines and condensed pyrimidines are summarized in Table 1.

The identity of products thus obtained was established primarily by the comparison of IR, UV, NMR and Mass spectra with those of authentic samples. With hitherto unknown compounds, the structure was established either by direct comparison with a sample obtained by an unequivocal synthesis (Chart1; IIb, VI) or by NMR and mass spectral data together with the elemental analysis.

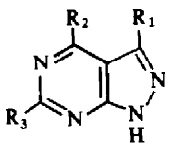
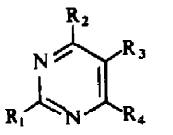
CHART 1



When methanol was used as solvent, the methylated product was the only product, but when ethanol or isopropanol was used the corresponding alkylated product was obtained in poor yield probably due to some unidentified side reactions. With 4-substituted pyrazolo[3.4-d]pyrimidines the methylation took place only at the 6-position, while with both 4-amino-5-cyanopyrimidine (VII) and 4-amino-5-amino-

† This paper was presented at the 88th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, 6 April 1968.

TABLE 1. PHOTO-INDUCED ALKYLATION OF PYRIMIDINES AND PYRAZOLO[3,4-d]PYRIMIDINES

	Starting material							Product					
	No.	R_1	R_2	R_3	R_4	Alcohol	Irradiation period (hr)	No.	R_1	R_2	R_3	R_4	Yield (%)
	III	H	OH	H		CH_3OH	6	IV[2]	H	OH	CH_3	—	67
	I	H	NH_2	H	-	CH_3OH	6	II_a[3]*	H	NH_2	CH_3	—	45
	I	H	NH_2	H	—	C_2H_5OH	6	II_b	H	NH_2	C_2H_5	—	41
	I	H	NH_2	H	—	iso-C_3H_7OH	10	II_c	H	NH_2	iso-C_3H_7	—	8
	V	CH_3	NH_2	H	—	CH_3OH	7	VI	CH_3	NH_2	CH_3	-	56
	VII	H	NH_2	CN	H	CH_3OH	6	VIII[4]	CH_3	NH_2	CN	CH_3	60
	IX	CH_3	NH_2	CN	H	CH_3OH	6	VIII[4]	CH_3	NH_2	CN	CH_3	86
	X	NH_2	NH_2	CN	H	CH_3OH	12	XI	NH_2	NH_2	CN	CH_3	57
	XII	H	NH_2	CH_2NH_2	H	CH_3OH	18	XIII[5]	CH_3	NH_2	CH_2NH_2	CH_3	52
	XIV	CH_3	NH_2	CH_2NH_2	H	CH_3OH	6	XIII[5]	CH_3	NH_2	CH_2NH_2	CH_3	33

Irradiated with a 40-W low-pressure mercury lamp in alcohols containing 2% hydrogen chloride.

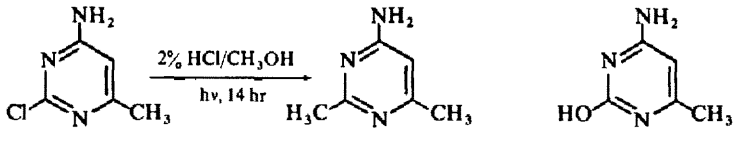
* II_a was also obtained by prolonged irradiation with a high-pressure mercury lamp or sunlight.

methylpyrimidine (XII) the corresponding 2,6-dimethylated compounds were obtained. It is obvious from the comparison of the yields shown in Table 1 that pyrimidines bearing an electron-withdrawing group at the position were more susceptible to methylation—VII gives VIII in 60% yield after 6 hr irradiation, while XII gives XIII in 52% yield after 18 hr irradiation; IX gives VIII in 86% yield, while XIV gives XIII in 33% yield after 6 hr irradiation, respectively.

Under similar conditions, 2-mercapto-4-hydroxy-6-aminopyrimidine (XV) and 2,6-dimethyl-4-aminopyrimidine (XVI) were recovered unchanged and 2-methyl-4-hydroxy-5-cyanopyrimidine (XVII), 2-methyl-4-hydroxy-5-aminomethylpyrimidine (XVIII), 2-amino-4-chloro-6-methylpyrimidine (XIX), cytosine (XX) and uracil resulted in the disappearance of the UV absorption. The addition of methanol to the C_5—C_6 double bond[6] or the cleavage of the pyrimidine ring[7] may have occurred during the reaction with some of these pyrimidines.

2-Chloro-4-amino-6-methylpyrimidine (XXI, isomer of XIX) afforded 2,6-dimethyl-4-aminopyrimidine (XVI) and 6-methylcytosine (XXII) in 19·5% and 64% yields,

CHART 2



XXI XVI XXII

respectively. In this reaction the methylation occurred at the position originally chlorinated.† The identity of XVI was established by the elemental analysis and the comparison of NMR and mass spectra with those of an authentic sample.[9]

In order to examine the effect of other acids on the present reaction, 4-amino-5-cyanopyrimidine (VII) was irradiated in methanol containing 2% (w/w) of various acids. No significant difference was noted except in the case where perchloric acid was used as judged by paper chromatography (Fig. 1.).

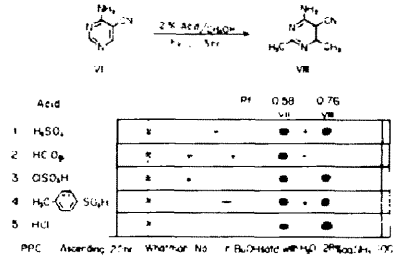


FIG. 1 Paper chromatogram of irradiated mixture.

† A similar reaction has been reported.[8]

The effect of the quantity of acid on this reaction was examined by irradiating (i) a solution of 2-methyl-4-amino-5-cyanopyrimidine (IX) in methanol, (ii) a solution of IX in methanol containing 2% of hydrogen chloride and (iii) a solution of the hydrochloride of IX in methanol. The reactions were checked with lapse of time by paper chromatography, and in all cases it was found that the reaction proceeds almost equally and IX was completely converted to 2,6-dimethyl-4-amino-5-cyanopyrimidine (VIII) within 6 hr. The UV spectrum of IX is shown in Fig. 2.

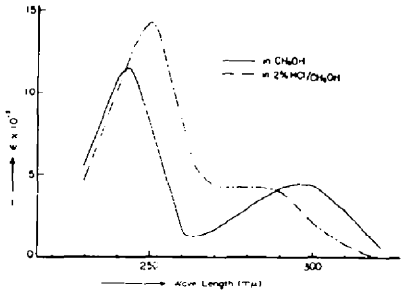


FIG. 2 UV absorption spectra of 2-methyl-4-amino-5-cyanopyrimidine (IX).

If the material is dissolved in methanol, the present photo-induced methylation gives the same product regardless of the quantity or the presence of an acid. This suggests that the π–π^* state of the base probably participates in the reaction.[7]

We attempted to calculate the reactivity indices by the MO method and correlate the calculated values with the experimental results which are summarized in Table 1. Since the present reaction is most probably a free radical reaction and the reactivities in a free radical mechanism can be reflected by either the superdelocalizability or the frontier electron density on a given reaction center of the molecule, we calculated these indices together with other characteristics by the ω-technique[10] ($\omega = 0{\cdot}55$[11]). The parameters[12] employed for the calculation and the reactivity indices are shown in Tables 2 and 3, respectively.

TABLE 2. PARAMETERS USED FOR CALCULATION BY THE ω-TECHNIQUE

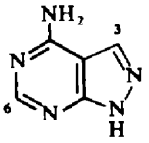
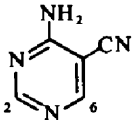
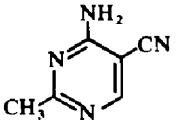
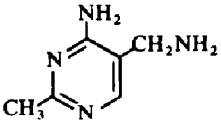
C—X	a_X*	a_C†	l‡	Ref.
C=N—	0·6	0·1	1	12
C—N	1	0·1	1	12
C—NH_2	0·4	0	0·6	12
C—CH_3	3	−0·1	1	12
C≡N	0·8	0	2	
C—CN	—	—	0·8	
C—CH_2NH_2	—	—	0·7	
C=H_2	−0·5	−0·1	2·5	

* Coulomb integral of the substituent X: $\alpha_X = \alpha + a_X\beta$

† Coulomb integral of the carbon atom adjacent to X: $\alpha_{adj} = \alpha + a_C\beta$.

‡ Resonance integral of the carbon atom and X: $\beta_{C-X} = l\beta$.

TABLE 3. REACTIVITY INDICES CALCULATED BY THE ω-TECHNIQUE

	I		VII		IX	XIV
	C-3	C-6	C-2	C-6	C-6	C-6
$Sr^{(R)}$	0·9025	0·9196	0·9241	0·9233	0·9152	0·8991
$fr^{(R)}$	0·1151	0·1958	0·2808	0·2297	0·3902	0·3504
Fr	0·4332	0·4064	0·4053	0·4216	0·4204	0·4146
Fr_1	0·5121	0·5707	0·6672	0·6130	0·7709	0·7335
ΔE	1·0829 β		1·1641 β		1·2189 β	1·1462 β
Fr_2	0·4428	0·5759	0·4968	0·5600	0·4219	0·4327
ΔE	1·3859 β		1·2570 β		1·3183 β	1·3097 β
ρ	1·0395	0·9490	0·9307	0·9380	0·9312	0·9478

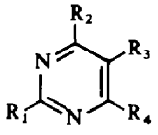
$Sr^{(R)}$: Superdelocalizability for radical reaction.
$fr^{(R)}$ Frontier electron density for radical reaction.
Fr: Free valence for ground state.
Fr_1: Free valence for one-electron excited state from the highest occupied orbital to the lowest vacant orbital.
Fr_2: Free valence for one-electron excited state from the highest occupied orbital to the 2nd lowest vacant orbital.
ΔE: Energy difference between these excited states and the ground state.
ρ Electron density.

From Table 3 one may assume that 4-aminopyrazolo[3.4-d]pyrimidine (I) is more susceptible to the radical reaction at the 6-position than at the 3-position and this proved to be the case as described in the experimental result shown in Table 1. With compound VII, which has no substituent on either the 2- or 6-position, the difference of reactivity indices for both positions was too small to predict the more reactive positions. Actually, VII gave the dimethylated compound VIII. A comparison of the reactivity indices at the 6-position of 5-cyanopyrimidine derivative (IX) with those of 5-aminopyrimidine derivative (XIV) indicates that the former is more reactive. This was in accord with the experimental data as judged from the methylation yields of both compounds. Table 3 also indicates that the values of free valence for excited states (Fr_1 and Fr_2) show a fairly good correlation with the values obtained with both superdelocalizability and frontier electron density calculations, whereas the values of free valence for the ground state (Fr) are very different from the aforementioned values. Table 3 also indicates that the current photo-induced methylation occurs preferentially at the position where the electron density is low.

Apparently, the Me group introduced by this reaction arises from the methanol used as solvent. Although the photolysis of methanol itself has been investigated,[13] the photolysis of methanol under the conditions in the present reaction are unknown.

Accordingly, we examined the ESR spectra of methanol with or without 2% amount of hydrogen chloride in the presence of pyrimidine compounds and others immediately after 1 hr of irradiation at the temperature of a liquid nitrogen. The results are summarized in Table 4.

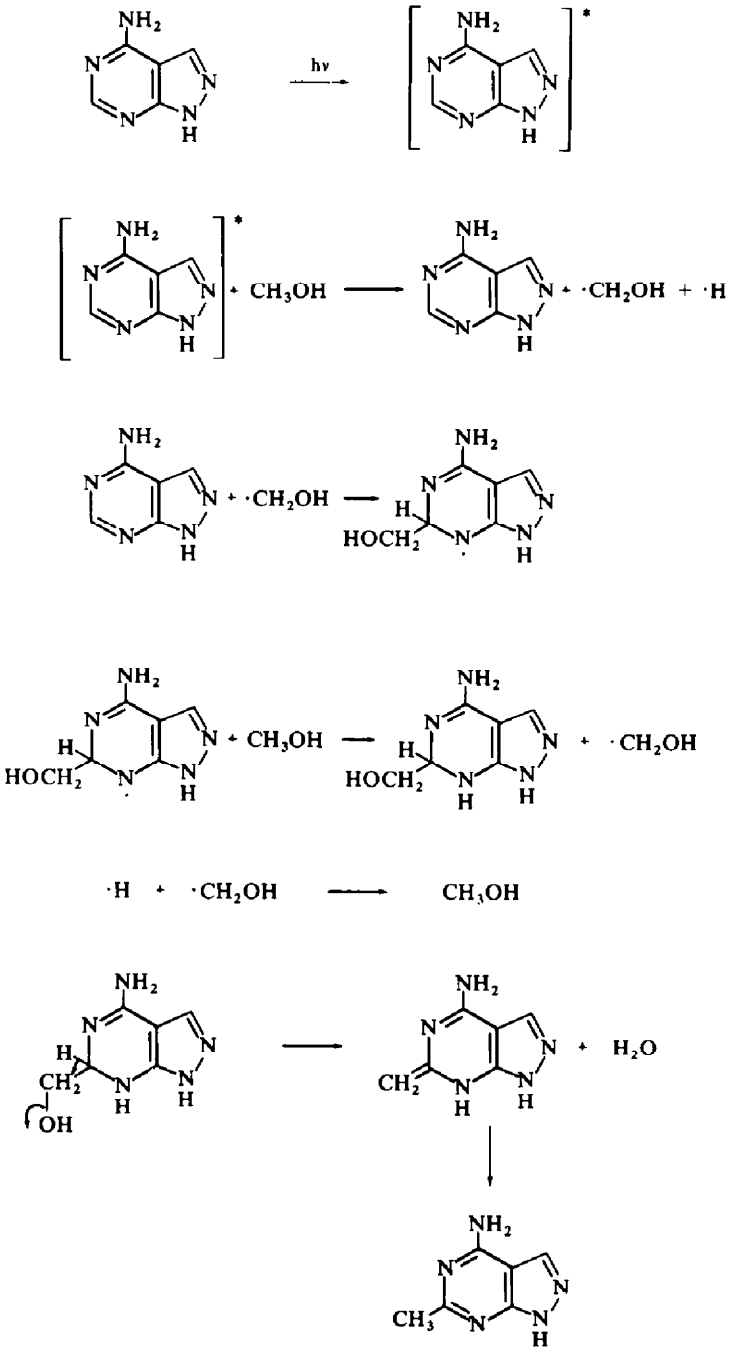
TABLE 4. RELATIVE INTENSITIES OF ESR SPECTRA OF METHANOL IRRADIATED WITH LIGHT (250–600 mμ) FOR 1 HR AT 77°K IN THE PRESENCE OF 1% INDUCERS AND 2% HYDROGEN CHLORIDE*

Inducers	R_1	R_2	R_3	R_4	No.	$\cdot CH_3$	$\cdot CH_2OH$	$\cdot CHO$
None						1	1	4
4-Aminopyrazolo[3.4-d]pyrimidine					I	0	7	2
Adenine						0·2	1	1
	CH_3	NH_2	CN	H	IX	1	13	4
	CH_3	NH_2	CH_2NH_2	H	XIV	1	18	18
	CH_3	OH	CN	H	XVII	1	6	3
	CH_3	OH	CH_2NH_2	H	XVIII	4	6	8
	Cl	NH_2	H	CH_3	XXI	0·4	14	3
	OH	OH	H	H		0·1	0·6	0·6
	OH	NH_2	H	H	XX	0	1	0·7
	SH	NH_2	H	OH	XV	0	0·4	0
Non + Glass Filter						0	0·5	0
IX + Glass Filter						0	0·6	0
XIV + Glass Filter						0	0·7	0
Benzene						3	21	52
Naphthalene						1	19	18
Biphenyl						3	20	23
Aniline						8	22	70
Diphenylamine						0	137	24
Cyclohexylamine						1	1	10

* Similar photolysis pattern was observed in the absence of hydrogen chloride in case the inducer was soluble in methanol.

The photolysis of methanol in the absence of pyrimidines gave essentially the same results as reported by Sullivan *et al.*[13] and the addition of 2% of hydrogen chloride did not change the photolysis pattern, but the addition of pyrimidines caused a remarkable increase in the concentration of $\cdot CH_2OH$. This tendency was especially remarkable with the bases such as I, IX and XIV, all of which are prone to the photomethylation. Contrary to this, cytosine (XX) and uracil which are insusceptible to the methylation exerted little effect on the increase of $\cdot CH_2OH$. On the other hand, compounds XVII and XVIII, although they led to an increased $\cdot CH_2OH$ formation, resulted in unidentified compounds having no UV absorption. Several aromatic compounds such as aniline, diphenylamine, benzene, naphthalene and biphenyl also caused a remarkable increase of $\cdot CH_2OH$ formation but not with cyclohexylamine. When compounds IX and XIV in methanol containing 2% hydrogen chloride were irradiated across a glass filter which is not transparent to light with wavelengths shorter than 300 mμ, there was no effect on the enhancement of radical formation.

CHART 3



* Excited state

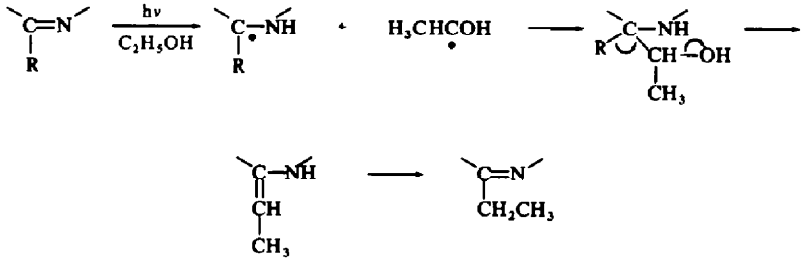
Smaller[14] reported that the rate of free radical production in the photolysis of methanol can be enhanced by addition of indole, but details of the mechanism were not given. Recently Kamiya *et al.*,[15] in their investigation of photolysis of methanol in the presence of *p*-benzoquinone, indicated that $\cdot CH_3$ and $\cdot CHO$ were formed after initial formation of $\cdot CH_2OH$.

These facts strongly suggest that some pyrimidine and aromatic compounds are first excited by irradiation and the excited species then induce the photolysis of methanol resulting in formation of the $\cdot CH_2OH$ radical.

From these considerations we postulate a possible mechanism for the present methylation reaction which is summarized as follows: a photo-excited pyrimidine (presumably π–π^*) abstracts hydrogen from methanol to give $\cdot CH_2OH$† and this radical then attacks the most reactive site of the pyrimidine, which may be foreseen in some cases from the reactivity indices calculated by the ω-technique, to establish the methylated pyrimidine as shown in Chart 3.

Recently Stermitz *et al.*[9] reported the incorporation of alcohol by irradiation of papaverine in alcohol to give 2-alkylisoquinoline derivatives. In a private communication‡ to the present authors they proposed the following mechanism (Chart 4).

CHART 4



R = H, Cl, Veratryl

EXPERIMENTAL

All m.ps are uncorrected. NMR spectra were measured with a Varian A-60 instrument with TMS as a standard. Mass spectra were measured with a Hitachi RMU-6D double focusing mass spectrometer.

2,4-*Diamino-5-cyano-6-methylpyrimidine* (XI). A soln of 135 mg of X in 400 ml MeOH containing 2% HCl was irradiated under N_2 with a 40-W low-pressure mercury lamp for 12 hr at room temp. During irradiation the reaction mixture was examined by TLC and a Uvicord set up with a column of cation-exchange resin (Dowex, 50 × 8, H^+ form). The reaction mixture was condensed under reduced press and the separated solid was recrystallized from aqueous MeOH to give colorless needles of XI hydrochloride, m.p. > 300°; yield, 105 mg; Mass M^+, 149; NMR (D_2SO_4); 2·64 ppm (s). (Found: C, 39·11; H, 4·44; N, 37·69. $C_6H_7N_5HCl$ requires: C, 38·80; H, 4·34; N, 37·73%).

2,4-*Dimethyl-5-cyano-6-aminopyrimidine* (VIII). A soln of 105 mg of IX in 400 ml MeOH containing 2%

† The participation of $\cdot CH_2OH$ in a photo-reaction of nitrogen-containing heterocyclic compounds in methanol has been reported.[16]

‡ August 30, 1967.

HCl was irradiated under N_2 for 6 hr. The reaction was condensed under reduced press and to the condensate 10 ml water was added. The acidic soln was run through a column packed with 3 g active charcoal. After being washed with water, the column was eluted with a mixture of solvents (water–MeOH–BuOH–28% NH_4OH = 2:6:1:1). From the eluate, after evaporation, 100 mg of colorless powder was obtained. The identity was confirmed by the comparison of IR spectrum with that of an authentic sample.

2,4-*Dimethyl-6-aminopyrimidine* (XVI) *and* 2-*hydroxy-4-amino-6-methylpyrimidine* (XXII). A soln of 717·5 mg of XXI in 800 ml MeOH containing 2% HCl was irradiated under N_2 for 14 hr. The reaction mixture was condensed under reduced press until a crystalline substance separated. The condensate was cooled in an ice-bath and the separated substance was collected by suction—400 mg XXII; Mass; M^+, 125; NMR (D_2O); 2·35 ppm (s, 3H), 6·03 ppm (s, 1H). The identity was confirmed by the comparison of IR spectrum with that of an authentic sample.[17] To the filtrate was added 10 ml water and the acidic soln was treated with active charcoal as described above. Chromatography of the powder thus obtained on silica gel using $CHCl_3$:acetone:EtOH (30:70:5) as solvent yielded XVI as colorless crystalline substance (120 mg); Mass; M^+, 123; NMR (D_2O); 2·20 ppm (s, 3H), 2·32 ppm (s, 3H), 6·20 ppm (s, 1H). The identity was confirmed by the comparison of NMR and Mass spectra with those of an authentic sample,[8] and the elemental analysis.

4-*Amino-6-isopropylpyrazolo*[3.4-d]*pyrimidine* (IIc). A soln of 270 mg of I in 600 ml isopropanol containing 2% HCl was irradiated under N_2 for 10 hr. The reaction mixture was condensed under reduced press to about 5 ml and 10 ml water was added. From this soln, after treatment with active charcoal, 262 mg of powder was obtained. Chromatography of this powder on silica gel using $CHCl_3$:acetone:EtOH (60:40:4) as solvent yielded IIc as colorless powder (30 mg), m.p. 256·5°; Mass; M^+, 177; NMR (d_6-$DMSO_4$); 1·25 ppm (d, J = 6·5 cs, 6H), 2·38 ppm (qu, J = 6·5 cs, 1H), 7·98 ppm (s, 1H). (Found: C, 54·07; H, 6·69. $C_8H_{11}N_5$ requires: C, 54·22; H, 6·26%).

Other photo-induced methylations were carried out in a similar fashion.

3,6-*Dimethyl-4-aminopyrazolo*[3,4-d]*pyrimidine* (VI). To a soln of 280 mg metallic Na in 25 ml EtOH were added 1·1 g acetamidine hydrochloride and 1·22 g 3-methyl-4-cyano-5-aminopyrazole.[18] The mixture was refluxed for 2·5 hr. After cooling, the separated solid was collected by suction and recrystallized from MeOH to give colorless needles (0·5 g), m.p. > 300°. (Found: C, 51·62; H, 5·73; N, 42·89. $C_7H_9N_5$ requires: C, 51·52; H, 5·56; N, 42·92%). The IR spectrum confirmed the identity with the sample obtained by photo-methylation.

4-*Amino-6-ethylpyrazolo*[3.4-d]*pyrimidine* (IIb). To 10 ml MeOH containing 10% NH_4OH, 432 mg 4-cyano-5-aminopyrazole[19] and 330 mg propionitrile were added. The reaction mixture was heated at 200° in a sealed tube for 20 hr and then condensed under reduced press and 20 ml 5% HCl was added to the condensate. Chromatography of the soln on a cation-exchange resin (IR-120, H^+ form) using 5% HCl as solvent followed by treatment with charcoal gave a powder. Chromatography of this powder on silica gel using $CHCl_3$–MeOH (9:0·75) as solvent yielded IIb as colorless crystals (30 mg), m.p. 257°; Mass; M^+, 163. (Found: C, 51·00; H, 5·38; N, 42·79. $C_7H_9N_5$ requires: C, 51·52; H, 5·56; N, 42·92%). The IR spectrum was identical with that of the sample obtained from I by the photo-induced ethylation.

Measurement of ESR. Samples of MeOH with or without 1% of inducers and 2% HCl were placed in quartz tubes, 3·5 mm i.d., which were irradiated for 1 hr with the light at 250–580 mμ from a high-pressure mercury lamp, Toshiba's SHL 100UV, at a distance of 12 cm in a liquid-N_2-filled quartz Dewar, 7 mm i.d. The ESR spectrometer used was a X-band instrument, manufactured by the Japan Electron Optics Co., Model JES-3B type; it employs a multipurpose cavity TE 102, 100 kc/s field modulation with 5·8 gauss in width and an electromagnet with 30 cm ϕ pole pieces. The ESR measurement was conducted immediately after irradiation at a liquid N_2 temp for 1 hr.

The Me radical (·CH_3) gave a quartet with an intensity ratio of 1:3:3:1, a width at maximum slope (ΔH msl) of 4 gauss, and a coupling constant (a) of 23 gauss. The formyl radical (·CHO) showed an asymmetrical doublet with (a) of 125 gauss and ΔH msl of 8 gauss. The hydroxymethyl radical (·CH_2OH) afforded a triplet with an intensity ratio of 1:2:1, (a) of 18 gauss and ΔH msl of 12 gauss. The relative intensities of the radicals shown in Table 4 were calculated by the sum of the peak-to-peak distance corrected for the attenuation.

The spectrum of ·CH_3 decreased rapidly even at 77°K, whereas the spectra of ·CH_2OH and ·CHO were stable for a few days although all the signals disappeared rapidly at room temp. Aqueous solutions of *N*HCl with or without inducers (I, IX, XIV) gave only a very weak signal probably due to ·OH radical on the irradiation at 77°K.

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