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Methylation of Some Deprotonated Sterically Hindered Pyrimidin-4-ols*

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Abstract

Anions derived from t-butyl-substituted pyrimidin-4-ols were methylated with iodomethane. The site of methylation was determined by proton-coupled ^{13}C n.m.r. and the relative proportions of isomers were determined by ^{1}H n.m.r. A t-butyl substituent *ortho* to a ring nitrogen markedly reduced the propensity for methylation at that nitrogen to the point where *O*-methylation, uncommon under these conditions, was observed.

Introduction

The biological importance of heteroaromatic tautomerism has been the subject of several reports[1] which indicate that the minor tautomeric form of some nucleic acid bases determines the frequency of mutations. The tautomerism of heteroaromatic compounds has recently been the subject of a review by Katritzky *et al.*[2] At biological pH some of these compounds exist in the anionic form, and hence the corresponding mesomeric or canonical forms of the anion become important. However, reactions of these anions rely not so much on the bias of the resonance 'equilibrium' as on the reactivity of the various mesomers. Such reactivity can often be rationalized by the 'hard–soft' reagent argument.

Diazomethane has been known to *O*-methylate pyrimidin-2- and -4-ols in low yield,[3] and this has been used in the past, incorrectly, as evidence for the hydroxyl structure of barbituric acid.[4] However, the attempted methylation of pyrimidin-2- and -4-ol sodium salts by iodomethane (the Williamson synthesis of ethers) typically yields only *N*-alkylated products. In fact even pyrimidin-5-ols, which are true phenolic substances, do not *O*-methylate satisfactorily with iodomethane.[5]

* Throughout this paper, such names as 'pyrimidin-4-ol' will be used without implying that the tautomer with an OH group is necessarily present in more than a trace quantity. Pyrimidin-4-ols and pyrimidin-6-ols are known to exist predominately in the oxo form in solution.

[1] Topal, M. D., and Fresco, J. R., *Nature (London)*, 1976, **263**, 285; Langlet, J., Claverie, P., Caron, F., and Boeuve, J. C., *Int. J. Quantum Chem.*, 1981, **20**, 299.
[2] Katritzky, A. R., Karelson, M., and Harris, P. A., *Heterocycles*, 1991, **32**(2), 329.
[3] David, S., and Hirshfeld, H., *Bull. Soc. Chim. Fr.*, 1966, 527.
[4] Wood, J. K., and Anderson, E. A., *J. Chem. Soc.*, 1909, **95**, 979; Arndt, F., *Rec. Fac. Sci. Univ. Istanbul*, 1936, **1**, 1 (*Chem. Abstr.*, 1937, **31**, 1006).
[5] Brown, D. J., 'The Pyrimidines' p. 248 (Interscience: New York 1962).

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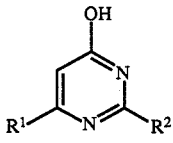
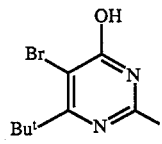
Jonak *et al.*[6] described the effects of the alkylating agent, cation, and solvent, when alkylating the sodium salts of pyrimidin-4-ols. They found that treating the sodium salt of 2-methylpyrimidin-4-ol with iodomethane in various solvents led exclusively to *N*-methylated products (N 1 and N 3). However, by increasing the bulk of both the electrophile, and the substituent in the 2-position, oxygen alkylation occurred at the expense of N 3 alkylation. These results were rationalized on the grounds that nitrogen alkylation is decreased due to steric effects while the rate of oxygen alkylation remains fairly constant.

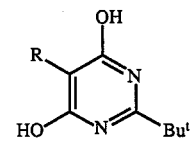
Because of our interest in sterically constrained pyrimidines, we possessed some t-butyl-substituted pyrimidinols (1)–(7). We decided to investigate the behaviour of the sodium salts of these compounds towards methylation with iodomethane.



	R^1	R^2
(1)	Bu^t	H
(2)	H	Bu^t
(3)	Bu^t	Bu^t



(4) R = Bu^t
(5) R = H



(6) R = H
(7) R = Bu^t

Results and Discussion

The parent pyrimidinols were deprotonated with sodium methoxide in methanol, and then stirred with 1·1 mol. equiv. of iodomethane for 72 h. The reaction products were examined by t.l.c., g.l.c., and proton n.m.r. spectroscopy in order to determine the extent of reaction, the number of methylation products, and their relative proportions (Table 1). In each case the t.l.c., g.l.c., and proton spectrum of the reaction mixture were consistent with there being only one or at the most two isomeric methylation products. The resonance of the methyl group protons of the methoxypyrimidine in each case appeared at lower field in proton n.m.r. than that of the methyl group protons of the corresponding isomeric *N*-methylated pyrimidine.

Where the reaction did not go to completion, starting material was recovered to make up the mass balance. The detection level of methylation isomer ratios, by using a combination of t.l.c., g.l.c., and proton n.m.r. analyses, is estimated to be in the order of ±1%.

Table 1. Relative percentage of methylation at oxygen and nitrogen, and ^{1}H and ^{13}C n.m.r. chemical shifts (ppm) of introduced methyl groups

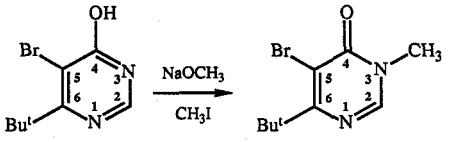
Compound methylated[A]	Relative percentage		Extent (%) of reaction	^{1}H chemical shift		^{13}C chemical shift	
	O-CH_3	*N*-CH_3		*O*-CH_3	*N*-CH_3	*O*-CH_3	*N*-CH_3
(1)	0	100	100	—	3·50	—	36·83
(2)	65	35	90	3·85	3·55	53·27	37·34
(3)	70	30	90	3·90	3·55	59·20	35·81
(4)	90	10	95	4·00	3·65	54·54	34·54
(5)	0	100	100	—	3·50	—	35·00
(6)	84	16	92	3·80	3·15	54·68	37·86

[A] Compound (7) did not methylate.

[6] Jonak, J. P., Hopkins, G. C., Minnemeyer, H. J., and Tieckelmann, H., *J. Org. Chem.*, 1970, **35**(8), 2512.

When two methylation isomers were present, they were successfully separated by column chromatography and distinguished from each other by techniques previously described.[7] In the ^{13}C–^{1}H coupled n.m.r. spectra of the *O*-methylated compounds, ^{3}J coupling was observed only between the carbon atom bearing the *O*-methyl group and the methyl protons. In contrast, with the *N*-methylated compounds further ^{3}J coupling of C 2 with the methyl protons was observed. The site of *N*-methylation can be unambiguously determined since, when methylation occurs at the nitrogen atom nearer to the hydroxy group, the carbon bearing the oxygen shows ^{3}J coupling to the methyl protons. This was observed in all cases, discounting the possibility of methylation at the remote ring nitrogen.

Thus, for example when 5-bromo-6-t-butylpyrimidin-4-ol (5) was methylated only one methylation product (*N*-$\mathbf{C}H_3$, 35·00 ppm) was obtained. From the ^{13}C–^{1}H coupled ^{13}C n.m.r. spectrum the site of methylation is clearly N 3.



(5) (8)

C 6 of (8) can be assigned (170·6 ppm) because of the multiplet (J 3·7 Hz) due to three-bond coupling to the t-butyl protons, C 5 appears as a singlet at its characteristic upfield shift (111·5 ppm), and C 2 can be identified from its large one-bond proton coupling of 205 Hz. The remaining downfield resonance (159·8 ppm) must, of course, be due to C 4. The C 2 and C 4 resonances both clearly show a ^{3}J quartet (J 4·4 Hz) due to the methyl group. For this to occur the methyl group must be attached to the N 3 site. Similar arguments were used to deduce the identity of all methylation isomers.

Barlin, Brown and Fenn recorded the ^{13}C n.m.r. spectra of various *N*- and *O*-methylated heteroaromtic compounds.[8] These authors found that the ^{13}C chemical shifts of the methoxy carbon atoms fell in the 53·20–61·87 ppm range while nuclear *N*-methyl carbon atoms appeared at 34·29–49·62 ppm. In our work all methylation products gave appropriate data within these ranges (Table 1); this confirmed our assignments.

The structures of the *O*-methylated compounds, deduced from spectroscopic evidence, were finally confirmed by their unambiguous synthesis. The parent pyrimidinols were first chlorinated with phosphoryl chloride, and then the chloropyrimidine was treated with sodium methoxide in methanol to yield the methoxypyrimidine in high yield. In each case these compounds were identical, with respect to i.r. and n.m.r., to the methylation product of lowest polarity in the two-component methylation mixtures.

It can be seen (Table 1) that the sterically demanding t-butyl group shields the annular nitrogen sites sufficiently to allow considerable reaction at oxygen. The presence of a bromine atom *ortho* to the hydroxy group, as in (4), further

[7] Jacobsen, N. W., and Rose, S. E., *Aust. J. Chem.*, 1985, **38**, 1809; Jacobsen, N. W., and de Jonge, I., *Aust. J. Chem.*, 1987, **40**, 1979.
[8] Barlin, G. B., Brown, D. J., and Fenn, M. D., *Aust. J. Chem.*, 1984, **37**, 2391.

enhances the reaction. However, the *ortho* bromo substituent without the ring nitrogen hindered by a t-butyl group, as in (5), fails to stimulate *O*-alkylation. This leads to the observation that, although electronic factors play a part, unless the ring nitrogen atoms are sterically hindered, reaction will take place there in preference to the oxygen functionality.

Since the attack on iodomethane by a nucleophile proceeds by an S_N2 mechanism, the nucleophile is attacking the carbon atom of a neutral molecule which is a relatively soft acid. The most electronegative atom of our mesomeric nucleophile is the harder base, and hence alkylation of the softer, or more polarizable, nitrogen atom of anions of pyrimdin-4-ol should predominate.[9]

In protic solvents, the most electronegative oxygen atom is better solvated and hydrogen-bonded. Therefore, in methanol, attack by the less electronegative nitrogen atom should be further enhanced. That such an extensive degree of *O*-methylation has been achieved with iodomethane in methanolic sodium methoxide in this work indicates that the products may be kinetically controlled to a large extent.

An *ortho* t-butyl group is an effective hindrance to ring nitrogen alkylation. 2,5-Di-t-butylpyrimidine-4,6-diol (7) did not methylate under the conditions employed, and this indicates that the t-butyl groups sterically hinder methylation at both sites. The importance of steric requirements for these and related reactions must always be taken into account, and may have relevance when considered with respect to their possible action in biological systems.

Experimental

General

Melting points were taken in open capillary tubes in an electrically heated silicon oil bath, and are uncorrected. High-resolution capillary gas chromatographic examinations were carried out with a Hewlett–Packard 5710-A gas chromatograph with a 25-m BP5 capillary column and nitrogen as carrier gas. Preparative gas chromatography was performed with a Shimadzu GC-9A gas chromatograph by using a 10% OV101 column. T.l.c. examinations were carried out on silica gel 60H with fluorescent indicator F_{254} for short-wavelength u.v. visualization. Infrared spectra were recorded on a PE 397 infrared spectrophotometer. Elemental analyses were performed by the University of Queensland Microanalytical Service. ^{1}H n.m.r. spectra were measured at 60 MHz with tetramethylsilane as an internal reference. ^{13}C n.m.r. spectra were recorded at 25·1 MHz in 10-mm tubes at concentrations of 80–150 mg/ml in $CDCl_3$ as solvent and internal lock, and with $Si(CH_3)_4$ as internal standard. The spectral window was 6 kHz with 16384 data points giving a digital resolution of better than 0·8 Hz/point. A pulse width of 8·8 μs (90°) was used with a pulse delay of 1·6 s and an acquisition time of 1·4 s. Good spectra were obtained after the accumulation of 2000-4000 free induction decays for decoupled spectra. Proton-coupled carbon-13 spectra were run on double precision with 32768 data points and a spectral window of 6 kHz. After the accumulation of *c.* 14000 free induction decays, spectra with a signal-to-noise ratio in excess of 100 were obtained.

2,6-Di-t-butylpyrimidin-4-ol,[10] 5-bromo-2,6-di-t-butylpyrimidin-4-ol,[11] 5-bromo-6-t-butylpyrimidin-4-ol,[11] 6-t-butylpyrimidin-4-ol,[12] 2-t-butylpyrimidin-4-ol,[13] 2-t-butylpyrimidine-4,6-diol[14] and 2,5-di-t-butylpyrimidine-4,6-diol[15] were prepared according to published procedures.

[9] Gompper, R., *Angew. Chem., Int. Ed. Engl.*, 1964, **3**, 560.
[10] Miller, G. W., and Rose, F. L., *J. Chem. Soc.*, 1963, 5642.
[11] Dox, A. W., *Org. Synth.*, 1948, Coll. Vol. I, 5.
[12] Rasmussen, C. A. H., and Plas, H. C. van der, *Recl Trav. Chim. Pays-Bas*, 1978, **97**, 288.
[13] Hymans, W. E., *J. Heterocycl. Chem.*, 1976, **13**, 1141.
[14] Helmkamp, G. K., and Kondo, N. S., *Biochim. Biophys. Acta*, 1968, **157**(2), 242.
[15] Evans, R. F., Savage, G. P., and Gough, D. A., *Aust. J. Chem.*, 1990, **43**(4), 733.

Methylations

Methylations were all performed on a 20 mM scale with 1·1 mol. equiv. of iodomethane and sodium methoxide in methanol (30 ml). The mixture was stirred at room temperature in a stoppered flask for 72 h. The resulting solution was evaporated to dryness and extracted with warm chloroform. The chloroform extracts were dried ($MgSO_4$) and concentrated under reduced pressure. Chromatography on Kieselgel 60 (230–400 mesh ASTM) with chloroform/ethyl acetate (4:1) as eluent in all cases afforded good separation of the different components, which sometimes included starting material as a minor component.

6-t-Butyl-3-methylpyrimidin-4(3H)-one

This compound was isolated as the sole methylation product of 6-t-butylpyrimidin-4-ol. It was recrystallized from ethanol, and subsequently sublimed (80°/0·2 mmHg) to give colourless *needles* with a melting point of 104° (Found: C, 65·7; H, 8·6; N, 16·8. $C_9H_{14}N_2O$ requires C, 65·0; H, 8·5; N, 16·8%). ^{1}H n.m.r. δ ($CDCl_3$) 1·25, s, $(CH_3)_3C$; 3·5, s, NCH_3; 6·4, s, H5; 8·1, s, H2.

2-t-Butyl-3-methylpyrimidin-4(3H)-one

The more polar isomer (R_F 0·5) of the two products from the methylation of 2-t-butylpyrimidin-4-ol was isolated as a white solid and then sublimed (95°/0·2 mmHg) to give *2-t-butyl-3-methylpyrimidin-4(3H)-one* which melted at 176° (Found: C, 65·4; H, 8·8; N, 16·9. $C_9H_{14}N_2O$ requires C, 65·0; H, 8·5; N, 16·8%). ^{1}H n.m.r. δ ($CDCl_3$) 1·3, s, $(CH_3)_3C$; 3·55, s, NCH_3; 6·4, d, J 6 Hz, H5; 8·3, d, J 6 Hz, H6.

2-t-Butyl-4-methoxypyrimidine

(A) This compound, the major product, was isolated from a methylation mixture and purified by column chromatography (R_F 0·8) to give a low-melting solid (Found: C, 65·2; H, 8·6; N, 16·6. $C_9H_{14}N_2O$ requires C, 65·0; H, 8·5; N, 16·8%). ^{1}H n.m.r. δ ($CDCl_3$) 1·4, s, $(CH_3)_3C$; 3·8, s, OCH_3; 6·5, d, J 6 Hz H5; 8·2, d, J 6 Hz, H6.

(B) 2-t-Butyl-4-chloropyrimidine (1·4 g, 8·2 mmol), prepared by the reaction between phosphoryl chloride and 2-t-butylpyrimidin-4-ol, was stirred at room temperature with a solution of sodium (0·2 g, 9 mmol) in dry methanol (30 ml) for 12 h. The solution was concentrated under reduced pressure and extracted with boiling chloroform to yield *2-t-butyl-4-methoxypyrimidine* (1·28 g, 94%) shown to be identical, by both i.r. and n.m.r. spectroscopy, with the specimen prepared in (A) above.

2,6-Di-t-butyl-3-methylpyrimidin-4(3H)-one

The minor but more polar product (R_F 0·4) of the two methylation isomers obtained from 2,6-di-t-butylpyrimidin-4-ol was isolated as a white solid and sublimed (60°/0·2 mmHg) to give *2,6-di-t-butyl-3-methylpyrimidin-4(3H)-one* with a melting point of 163° (Found: C, 69·2; H, 9·8; N, 13·3.* $C_{13}H_{22}N_2O$ requires C, 70·2; H, 10·0, N, 12·6%). ^{1}H n.m.r. δ ($CDCl_3$) 1·2, s, $(CH_3)_3C$; 1·3, s, $(CH_3)_3C$; 3·55, s, NCH_3; 6·15, s, H5.

2,4-Di-t-butyl-6-methoxypyrimidine

(A) This compound, the major methylation isomer, was isolated as a clear oil (R_F 0·9) and purified by preparative gas chromatography (Found: C, 69·2; H, 9·9; N, 13·3. $C_{13}H_{22}N_2O$ requires C, 70·2; H, 10·0; N, 12·6%). ^{1}H n.m.r. δ($CDCl_3$) 1·25, s, $(CH_3)_3C$; 1·5, s, $(CH_3)_3C$; 3·9, s, OCH_3; 6·4, s, H5.

(B) 2,4-Di-t-butyl-6-chloropyrimidine (1·5 g, 6·6 mmol), prepared by the reaction between phosphoryl chloride and 2,6-di-t-butylpyrimidin-4-ol, was stirred at room temperature with a solution of sodium (0·17 g, 7·3 mmol) in dry methanol (40 ml) for 15 h. After this period, the solution was concentrated under reduced pressure and extracted with boiling chloroform

* All methylation isomers were purified to a single peak on high-resolution g.l.c., but in some cases poor combustion analyses were still obtained.

to yield *2,4-di-t-butyl-6-methoxypyrimidine* (1·2 g, 81%) shown to be identical, by i.r. and n.m.r. spectroscopy, with the specimen isolated in (A) above.

5-Bromo-2,6-di-t-butyl-3-methylpyrimidin-4(3H)-one

The minor methylation product from 5-bromo-2,6-di-t-butylpyrimidin-4-ol was isolated by column chromatography (R_F 0·7) and recrystallized from ethanol. Sublimation (85°/0·5 mmHg) gave *5-bromo-2,6-di-t-butyl-3-methylpyrimidin-4(3H)-one* as a colourless solid which melted at 177° (Found: C, 52·2; H, 7·1; N, 8·9. $C_{13}H_{21}BrN_2O$ requires C, 51·8; H, 7·0; N, 9·3%). ^{1}H n.m.r. δ ($CDCl_3$) 1·4, s, $(CH_3)_3C$; 1·42, s, $(CH_3)_3C$; 3·65, s, NCH_3.

5-Bromo-2,4-di-t-butyl-6-methoxypyrimidine

(A) Evaporation of the combined appropriate chromatography fractions (R_F 0·95) gave a clear oil which was purified by repeated preparative gas chromatography until a single peak was observed upon high-resolution capillary gas chromatographic examination (Found: C, 52·1; H, 7·2; N, 8·9. $C_{13}H_{21}BrN_2O$ requires C, 51·8, H, 7·0; N, 9·3%). ^{1}H n.m.r. δ ($CDCl_3$) 1·4, s, $(CH_3)_3C$; 1·55, s, $(CH_3)_3C$; 4·0, s, OCH_3.

(B) 5-Bromo-2,4-di-t-butyl-6-chloropyrimidine (1·2 g, 3·9 mmol), prepared by treating 5-bromo-2,6-di-t-butylpyrimidin-4-ol with phosphoryl chloride, was stirred at room temperature with a solution of sodium (0·1 g, 4·3 mmol) in dry methanol (30 ml) for 15 h. The solution was concentrated under reduced pressure and the residue was then extracted with boiling chloroform to yield *5-bromo-2,4-di-t-butyl-6-methoxypyrimidine* (1·1 g, 93%) identical, by both i.r. and n.m.r. spectroscopy, with the sample in (A) above.

2-t-Butyl-6-hydroxy-3-methylpyrimidin-4(3H)-one

The white solid of higher polarity (R_F 0·6) between the methylation isomers obtained from 2-t-butylpyrimidine-4,6-diol was isolated and recrystallized from ethanol to give *2-t-butyl-6-hydroxy-3-methylpyrimidin-4(3H)-one* as white needles with a melting point of 173·5° (Found: C, 60·0; H, 8·1; N, 15·4. $C_9H_{14}N_2O_2$ requires C, 59·3; H, 7·7; N, 15·4%). ^{1}H n.m.r. δ ($CDCl_3$) 1·45, s, $(CH_3)_3C$; 3·15, s, NCH_3; 5·7, s, H 5.

2-t-Butyl-6-methoxypyrimidin-4-ol

This compound, the major isomer, was isolated and purified by column chromatography (R_F 0·8) to give a low-melting solid (Found: C, 59·8; H, 8·0; N, 15·3. $C_9H_{14}N_2O_2$ requires C, 59·3; H, 7·7; N, 15·4%). ^{1}H n.m.r. δ ($CDCl_3$) 1·4, s, $(CH_3)_3C$; 3·8, s, OCH_3; 5·6, s, H 5.

5-Bromo-6-t-butyl-3-methylpyrimidin-4(3H)-one

This compound was the sole methylation product from 5-bromo-6-t-butylpyrimidin-4-ol. *5-Bromo-6-t-butyl-3-methylpyrimidin-4(3H)-one* was isolated as a white solid and sublimed (75°/0·15 mmHg) to give colourless needles which melted at 165° (Found: C, 44·2; H, 5·6; N, 11·2. $C_9H_{13}BrN_2O$ requires C, 44·1; H, 5·4; N, 11·4%). ^{1}H n.m.r. δ ($CDCl_3$) 1·5, s, $(CH_3)_3C$; 3·5, s, NCH_3; 8·0, s, H 2.