one peak on vpc, and the nmr spectrum was identical with that of the analytical sample of the chloro compound **6** (prepared in another run).

The chloro compound **6** showed the following nmr spectrum in $CDCl_3$: 3.41 (t, J = 6 cps, CH_2Cl, 2 H), 3.3 (s, OCH_3, 3 H), 3.2–3.4 (m, OCH, 1 H), 0.8–2.3 (CH and CH_2, 11 H).

Anal. Calcd for $C_9H_{17}ClO$: C, 61.18; H, 9.63. Found: C, 61.38; H, 9.57.

cis-Perhydrobenzofuran (**5**) which was pure by vpc examination showed the following ir spectrum (cm^{-1}): 2940 (s), 2870 (s), 1445 (m), 1380 (w), 1360 (w, b), 1290 (w), 1240 (w), 1180 (w), 1155 (w), 1120 (w, b), 1080 (m), 1050 (m), 1025 (s), 1000 (w), 985 (m), 930 (w), 870 (w), 805 (w), 680 (w, b). The nmr spectrum in $CDCl_3$ showed 0.8–2.3 (b, 11 H), 3.6–4.2 (m, 3 H).

Reaction of *trans*-2-(2-Methoxycyclohexyl)ethanol (4) with Tosyl Chloride in Pyridine.—A mixture of 5 g of *trans*-2-(2-methoxycyclohexyl)ethanol, 6.3 g of tosyl chloride, and 44 ml of anhydrous pyridine was stirred for 5.5 hr at 63–65°, poured onto 70 g of cracked ice, and extracted with ether (seven 20-ml portions). The usual work-up gave 2.3 g of crude product. Distillation at 7 mm gave the following cuts: (1) bp 75–90°, 0.1 g; (2) bp 93–95°, $n^{25}D$ 1.4650, 1.7 g (30%). Vpc analysis of 1 showed a mixture of the unsaturated compound **8**, and the *trans*-chloro compound **7**. Fraction 2 showed only one peak, the chloro compound **7**; the nmr spectrum of this compound (taken in CCl_4 on a sample from a different run) showed 1.5 (m, 11 H), 2.8 (m, $CHOCH_3$), 3.22 (s, OCH_3, 3 H), and 3.45 (t, J = 6.5 cps, CH_2-CH_2Cl, 2 H).

Anal. Calcd for $C_9H_{17}ClO$: C, 61.18; H, 9.63; Cl, 20.06. Found: C, 61.31; H, 9.68; Cl, 19.76.

The structure of the unsaturated compound **8** was based on the following nmr spectrum ($CDCl_3$): 3.27 (s, OCH_3, 3 H), 3.7 (m, CHO, 1 H), 4.7–5.7 (m, vinyl H, 3 H). Ir bands appeared at 3090 and 1645 cm^{-1}.

Lactone of *trans*-(2-Hydroxycyclohexyl)acetic Acid (10b).—Coffey's procedure[7] was modified as follows. To a solution of 57 g of ethyl malonate and 9 g of sodium in 200 ml of absolute ethanol was added 33 g of cyclohexene oxide in 100 ml of absolute ethanol. The reaction mixture became semisolid after reflux for a few minutes; reflux was continued for additional 30 min. Solvent was removed under vacuum. The residual semisolid material was dissolved in 200 ml of 10% NaOH, refluxed for 3 hr, concentrated under vacuum, acidified with HCl, and extracted with $CHCl_3$. The $CHCl_3$ layer was dried, solvent was removed, and the remaining oil was heated at 170–190° for 1 hr. Evolution of gas was observed. Distillation of resulting oil gave the lactone of cyclohexanolacetic acid, bp 97–98° (2 mm), C=O band at 1785 cm^{-1}.

***trans*-2-(2-Hydroxycyclohexyl)ethanol (11).**—To a suspension of 1.8 g of lithium aluminum hydride in 50 ml of ether was added dropwise 6.1 g of the lactone **10b**; the mixture was stirred for 30 min at 0° and then for 4 hr at room temperature. Work-up in the usual way and distillation yielded 4.1 g of the diol **11** as a viscous colorless liquid, bp 104–105° (1 mm). A sample was treated with bis(trimethylsilyl)trifluoroacetamide; vpc showed a single peak. The ir (liquid film) showed bands at 3300 (b), 1450, 1070, 1055, and 1035 cm^{-1}. The nmr in $CDCl_3$ showed 0.9–2.2 (m, CH_2 and CH, 11 H), 2.9–3.4 (m, 1 H), 3.5–3.8 (m, 2 H), 4.7 (OH, 2 H).

***trans*-Perhydrobenzofuran (9).**—The trans diol **11** (3.3 g) was heated with 6.6 g of tosyl chloride in 35 ml of dry pyridine at 95–100° for 2 hr. Distillation of the product resulting from the usual work-up gave 2.4 g of the *trans*-perhydrobenzofuran, bp 72° (25 mm), $n^{25}D$ 1.4632. This material was homogeneous when examined by vpc; addition of a pure sample of *cis*-perhydrobenzofuran (**5**) showed two peaks. Molecular weight by mass spectroscopy was 126 (calcd 126). The nmr spectrum in $CDCl_3$ showed 0.8–2.3 (11 H), 3.6–4.2 (3 H). The ir in liquid film showed the following bands, clearly different from the cis compound above: 2940 (s), 2870 (s), 1455 (m), 1390 (w), 1355 (w), 1340 (w), 1308 (w), 1290 (w), 1270 (w, b), 1190 (w), 1145 (m), 1110 (w), 1065 (s), 1055 (sh), 1020 (w), 980 (s), 930 (m), 925 (sh), 915 (sh), 857 (m), 830 (w), 660 (w, b). These properties supplant those previously reported by us.[2]

Registry No.—**3**, 27345-66-0; **4**, 27345-67-1; **5**, 10198-29-5; **6**, 27384-94-7; **7**, 27345-69-3; **9**, 27345-70-6; **10b**, 27345-71-7; **11**, 27345-72-8.

Reactivities and Electronic Aspects of Nucleic Acid Heterocycles. II. Diazomethane Methylation of Uracil and Its Methyl Derivatives[1]

JOHN L. WONG* AND DAVID S. FUCHS

Department of Chemistry, University of Louisville, Louisville, Kentucky 40208

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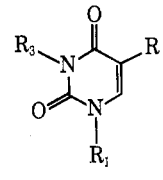
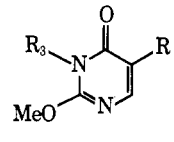
The methylation with diazomethane of nucleic acid constituents has been extensively studied[2] in connection with the plausible relationship[3] between the mechanism of mutagenesis and carcinogenesis. The action of diazomethane on uracil (**1**) and thymine (**1a**) was reported earlier[4] to afford only the 1,3-dimethyl derivatives, and reaction of diazomethane with uridines or the uracil residue in dinucleoside phosphates yielded exclusively the 3-N-methylation products.[5] However, in the case of diazomethane methylation of 1-β-D-arabinofuranosyl-5-fluorouracil, a minor amount of 4-O-methylation was also observed.[6]

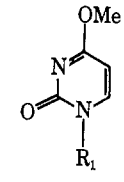
We have found that uracil (**1**), upon treatment with diazomethane, gave rise to four dimethyl compounds: 1,3-dimethyluracil (**2**), 2-methoxy-3-methyl-4-pyrimidone (**3**), 4-methoxy-1-methyl-2-pyrimidone (**4**), and 2,4-dimethoxypyrimidine (**5**). These products were isolated by preparative thin layer and gas–liquid phase



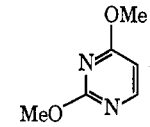
1, R = R_1 = R_3 = H
1a, R = Me; R_1 = R_3 = H
2, R = H; R_1 = R_3 = Me
6, R = R_1 = H; R_3 = Me
6a, R = R_3 = Me; R_1 = H
7, R = R_3 = H; R_1 = Me



3, R = H; R_3 = Me
3a, R = R_3 = Me
8, R = R_3 = H



4, R_1 = Me
9, R_1 = H



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chromatography. The previously unreported dimethyluracil (**3**) was identified by its nmr spectrum

(1) Support of this work by the Public Health Service Grant CA-10142 is gratefully acknowledged.

(2) (a) T. Ueda and J. J. Fox, *Advan. Carbohyd. Chem.*, **22**, 382 (1967); (b) R. L. C. Brimacombe, B. E. Griffin, J. A. Haines, W. J. Haslam, and C. B. Reese, *Biochemistry*, **4**, 2452 (1965).

(3) P. N. Magee and J. M. Barnes, *Advan. Cancer Res.*, **10**, 227 (1967).

(4) F. C. Case and A. J. Hill, *J. Amer. Chem. Soc.*, **52**, 1536 (1930).

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TABLE I

REACTION OF DIAZOMETHANE WITH URACIL AND ITS METHYL DERIVATIVES[a]

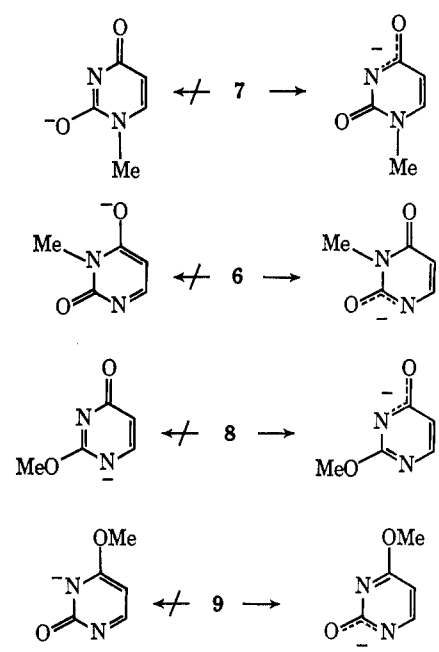
	Acidic pK_a[c]	$\nu_{C=O}^{KBr}$, cm^{-1}	Yield, %	Methylation products, % N^1,N^3	N^3,O^2	N^1,O^4	O^2,O^4	N:O ratio
Uracil (1)	9.5	1695 1630	98	73	18	4	5	3.5
Uracil (1)[b]			48	65	19	6	10	2.6
1-Methyluracil (7)	9.75	1695 1650	91	91		9		10.1
3-Methyluracil (6)	9.95	1690 1665	82	72	28			2.6
2-Methoxy-4-pyrimidone (8)	*Ca.* 8.2	1620	62		86		14	6.1
4-Methoxy-2-pyrimidone (9)	*Ca.* 10.7	1630	64			61	39	1.6
Thymine (1a)	9.9	1720 1660	99	69	20	6	5	3.1

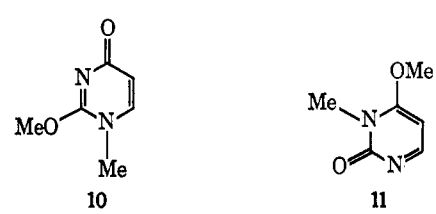
[a] The uracils (0.1 mmol) in 1 ml of methanol were stirred with 30 ml (3.5 mmol) of ethereal diazomethane at room temperature overnight. [b] Dimethylformamide replaced methanol in previous conditions. [c] D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, 9, 199 (1952); pK_a values for 8 and 9 are estimated from those reported for the corresponding ethoxy derivatives.

[($CDCl_3$) δ 3.42 (s, 3, NMe), 4.03 (s, 3, OMe), 6.17 (d, 1, J = 6 Hz, 5-H), and 7.65 (d, 1, J = 6 Hz, 6-H)] and uv absorption [$\lambda_{max}^{H_2O}$ pH 7.4 269 nm (ϵ 6130) and 213 (4340)], which is comparable to that of 2-ethoxy-3-methyl-4-pyrimidone.[7] Final proof of the structure was accomplished by hydrolysis of **3** in refluxing 1 *N* hydrochloric acid to give 3-methyluracil (**6**) and amination in methanolic ammonia at 100° to produce 3-methylisocytosine.[8] The percentages of the dimethyl isomers were quantitated by glpc using authentic samples as standards for calibration. The results are shown in Table I. The methylation of **1** experienced a significant solvent effect yielding greater amounts of O-methylation products in the medium of dimethylformamide–ether (1:30) than in methanol–ether (1:30). The respective N:O methylation ratios are 2.6 and 3.5 as shown in Table I. Similar solvent effect on the N:O methylation ratio has been noted for the reaction of saccharin with diazomethane.[9] Table I also reveals a methylation pattern for thymine (**1a**) resembling that of uracil (**1**). Apparently the steric and electronic effects on the course of methylation exerted by the 5-methyl group of **1a** are not significant. The unknown 3,5-dimethyl-2-methoxy-4-pyrimidone (**3a**) was identified by comparing its uv and nmr spectra with those of **3** and its hydrolysis to 3-methylthymine (**6a**).

The pathways of methylation of uracil (**1**) were studied by treating the four monomethyluracils individually with diazomethane. Thus, 1-methyluracil (**7**) gave rise to dimethyluracils **2** and **4**, 3-methyluracil (**6**) to **2** and **3**, 2-methoxy-4-pyrimidone (**8**) to **3** and **5**, and 4-methoxy-2-pyrimidone (**9**) to **4** and **5**. All glpc components of the reaction mixtures were identified, and the percentage yields of the dimethyl compounds and the N:O ratios are shown in Table I. Since methylation occurs by substitution of a methyl group in place of the active lactam hydrogen, the selectivity of the action of diazomethane on the monomethyluracils can be rationalized in terms of plausible and implausible intermediate anions as shown in Scheme I. Thus, of the six possible dimethyluracils, 2-methoxy-1-methyl-4-pyrimidone (**10**) and 4-methoxy-3-methyl-2-pyrimidone (**11**) are not formed in these diazomethane reactions. The structure of 2-ethoxy-4-pyrimidone has been determined[10] recently to be predominantly in the *o*-quinonoid form in chloroform, *e.g.* **8**, and that of 4-alkoxy-2-pyrimidone has been routinely written[11] as **9**. The results of selective methylation of **8** and **9** with ethereal diazomethane tend to confirm these fine structural assignments.

SCHEME I





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(7) Ultraviolet spectrum of 2-*O*-ethyl-3-methyluracil [$\lambda_{max}^{H_2O}$ 216 nm (ϵ 3700), 271 (5900)]: M. Hirata, *Chem. Pharm. Bull.*, **16**, 430 (1968).
(8) D. J. Brown and N. W. Jacobsen, *J. Chem. Soc.*, 3172 (1962).
(9) R. Gompper, *Chem. Ber.*, **93**, 187, 198 (1960).
(10) J. Pitha, *J. Org. Chem.*, **35**, 903 (1970).
(11) (a) C. W. Noell and C. C. Cheng, *J. Heterocycl. Chem.*, **5**, 25 (1968); (b) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952).

A review article[12] on the methylation of lactams with diazomethane suggests that there is an intimate relationship between the position of the amide band and the orientation of methylation. Three major regions have been cited: (1) 1620–1680 cm^{-1}, O-methylation; (2) 1680–1720, O- and N-methylation with kinetic dependence; and (3) 1730–1800, N-methylation. The factual data in Table I indicates that such a correlation for uracil and its methyl derivatives is untenable. Also as illustrated in Table I, there is no apparent relationship between the acidic pK_a of uracil and the methyl derivatives and the yields or N:O ratios of the diazomethane reactions.

Experimental Section

Instrumentation and conditions for tlc and glpc analyses have been described in details in a related paper.[13] Melting points are uncorrected and microanalyses were performed by M-H-W Laboratories, Garden City, Mich. 48135.

Reaction of Uracil and Its Methyl Derivatives with Diazomethane.—To a mixture of 0.1 mmol of each of the pyrimidines **1**, **1a**, **6**, **7**, **8**, and **9** in 1 ml of anhydrous methanol was added 30 ml (3.5 mmol) of ethereal diazomethane. The solution was allowed to stir overnight when practically all the solid material had dissolved. Longer reaction time (48 hr) was allowed for uracil (**1**) when dimethylformamide instead of methanol was used. The solution was filtered, concentrated, and diluted to 1 ml volumetrically with methanol. Quantitative analyses by glpc for the four dimethyluracils were done by comparing their peak areas with those of the authentic samples on a 6 ft × 0.125 in. column packed with 10% Carbowax 20M on Anakrom ABS 60–70 mesh at the following conditions [T_I, T_C, T_D (°C)]: **2**, 250, 170, 260; **3**, 200, 120, 260; **4**, 250, 200, 260; and **5**, 200, 100, 260, and 30 cc/min of nitrogen. A homogeneous chromatogram was observed under the highest temperatures for all the dimethyluracil standard solutions [0.5 wt % in methanol and relative retention times (min) for **2**, **3**, **4**, and **5** are 10.0, 2.5, 18.0, and 1.0, respectively], except **4** showed a 7% rearrangement to **2**, and the yield of the latter in a methylation reaction was corrected accordingly. Under the various combination temperatures cited above, the methoxypyrimidones **8** and **9** were either retained or decomposed on the column. At the high temperature end, minor peaks identifiable as *N*-methyl- and dimethyluracils were seen whose areas accounted for <1% of the methoxypyrimidone injected.[14] The glpc properties of the methylthymines resemble those of the corresponding uracils and were analyzed in a similar manner. The results of the diazomethane reactions are summarized in Table I.

2-Methoxy-3-methyl-4-pyrimidone (3).—A mixture of 0.2 g (1.6 mmol) of 2-methoxy-4-pyrimidone (**8**)[13] in 5 ml of methanol was stirred with 30 ml (3.5 mmol) of ethereal diazomethane until the evolution of nitrogen had ceased. The solution was concentrated and chromatographed on a 9-g silica gel column with 25% ethyl acetate in chloroform as eluents, yielding 0.12 g (54%) of **3**. Recrystallization from anhydrous ether and sublimation (50°, 20 mm) gave a pure sample: mp 93–95°; uv $\lambda_{max}^{H_2O}$ 269 nm (ϵ 6130), 213 (4340) at pH 7.4; ir (KBr) 1635 cm^{-1} (C=O); nmr ($CDCl_3$) δ 3.42 (s, 3), 4.03 (s, 3), 6.17 (d, 1, J = 6 Hz), and 7.65 (d, 1, J = 6 Hz).

Anal. Calcd for $C_6H_8N_2O_2$: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.31; H, 5.81; N, 20.20.

3,5-Dimethyl-2-methoxy-4-pyrimidone (3a).—A mixture of 0.35 g (2.5 mmol) of 2-methoxy-5-methyl-4-pyrimidone[13] in 5 ml of methanol and 60 ml (7 mmol) of ethereal diazomethane was allowed to react, and the product was isolated as described for the preparation of **3**. Compound **3a**, 0.13 g (34%), was recrystallized from anhydrous ether and sublimed (50°, 20 mm): mp 106–108°; uv $\lambda_{max}^{H_2O}$ 272 nm (ϵ 6280), 217 (4630) at pH 7.4; ir (KBr) 1635 cm^{-1} (C=O); nmr ($CDCl_3$) δ 2.00 (d, 3, J = 1 Hz), 3.43 (s, 3), 4.00 (s, 3), 7.53 (q, 1, J = 1 Hz).

Anal. Calcd for $C_7H_{10}N_2O_2$: C, 54.54; H, 6.54; N, 18.17. Found: C, 54.54; H, 6.74; N, 18.41.

Registry No.—**1**, 66-22-8; **1a**, 65-71-4; **3**, 27460-04-4; **3a**, 27460-05-5; **6**, 608-34-4; **7**, 615-77-0; **8**, 25902-86-7; **9**, 18002-25-0; diazomethane, 334-88-3.

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(13) Part I: J. L. Wong and D. S. Fuchs, *J. Org. Chem.*, **35**, 3786 (1970).

(14) Pyrolysis of 4-methoxy-2-pyrimidone (**9**), 2 mg at 210–220° for 40 min in an evacuated tube, caused complete conversion to the following products identified by glpc and tlc: **1**, **2**, **4**, **5**, **6**, and **7**. Similar treatment of the 2-methoxy analog **8** yielded all of the above products plus **3**. In both cases, uracil (**1**) and the *N*-methyluracils **6** and **7** were the major products. For a reference to thermal-induced methyl migration of monomethoxypyrimidines, see D. J. Brown and T. C. Lee, *J. Chem. Soc. C*, 214 (1970), and refer to ref 13 for thermal and catalyzed isomerization of 2,4-dialkoxypyrimidines.

Evaluation of Acyloxysilane as an Acylating Agent for Peptide Synthesis

T. H. CHAN* AND L. T. L. WONG

Department of Chemistry, McGill University, Montreal, Canada

Received August 17, 1970

Few synthetic reactions have received more attention in recent years than that of the formation of the peptide linkage.[1] Many newer methods involving the use of ingeniously designed coupling reagents have been discovered.[2-4] Recently, in our laboratory, we found that[5] silicon tetrachloride can act as a simple and efficient coupling reagent for the formation of amide from carboxylic acid and amine according to eq 1. Be-

$$2RCO_2H + 2R'NH_2 + SiCl_4 \xrightarrow{\text{pyridine}} 2RCONHR' + (SiO_2)_n + 4HCl \quad (1)$$

cause of the ready availability of silicon tetrachloride and its apparent efficacy in mediating the formation of the amide bond, we have extended our investigation to the use of silicon tetrachloride as a coupling reagent for peptide synthesis.

Preliminary experiments indicated that the condensation between an N-protected amino acid and an amino ester with silicon tetrachloride did not yield the desired depeptide. While the N-protected amino acid could be recovered essentially quantitatively from the reaction mixture, the starting amino ester was converted into a polymeric material. Apparently, under the reaction conditions, a facile polymerization of the amino ester occurred. Similar observation was made by Birkofer[6] who found that polyglycine was obtained from the reaction of ethyl glycinate with silicon tetrachloride (reaction 2). Our task was therefore to minimize this side reaction.

$$n\text{-}NH_2CH_2CO_2C_2H_5 + (n/4)SiCl_4 \longrightarrow (\text{—}NHCH_2CO\text{—})_n + (n/4)Si(OC_2H_5)_4 \quad (2)$$

Results

Preliminary Studies.—Pertinent to the problem at hand are the following observations. Trimethylace-

(1) For a summary of reagents for peptide formation, see M. Bodanszky and M. A. Ondetti, "Peptide Synthesis," Interscience, New York, N. Y., 1966.

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(4) G. Gawne, G. W. Kenner, and R. C. Sheppard, *ibid.*, **91**, 5669 (1969).

(5) T. H. Chan and L. T. L. Wong, *J. Org. Chem.*, **34**, 2766 (1969).

(6) L. Birkofer and A. Ritter, *Justus Liebigs Ann. Chem.*, **612**, 22 (1958).