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Determination of the reactivity of uracil derivatives with respect to methyl iodide by high-performance thin-layer chromatographic densitometry

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ABSTRACT

A high-performance thin-layer chromatographic method is described for the investigation of the methylation rates of uracil, 5-fluoro-, 5-chloro-, 5-bromo-, 5-methyl- and 5-nitrouracil and their N^1- and N^3-methyl derivatives. The method allowed the amounts of compounds obtained, the time required to complete the reaction and therefore the reactivity of the uracil derivatives to be determined. The N^1- and N^3-methyl and $N^{1,3}$-dimethyl derivatives were synthesized and separated by droplet countercurrent chromatography and/or medium-pressure liquid chromatography and their R_F values were determined in different solvent systems by thin-layer chromatography.

INTRODUCTION

A high-performance thin-layer chromatographic (HPTLC) investigation into the reactivity with respect to methyl iodide of uracil, 5-fluoro-, 5-chloro-, 5-bromo-, 5-methyl- and 5-nitrouracil and their N^1-methyl and N^3-methyl derivatives in anionic form and tautomeric equilibrium (Table I) was carried out. For this purpose, uracil derivatives (**II, VI–VIII, X–XII, XV, XVI, XVIII–XX, XXII–XXIV**) were synthesized and separated by droplet countercurrent chromatography (DCCC) and/or medium-pressure liquid chromatography (MPLC) and their R_F values were determined in suitable solvent systems by TLC. An HPTLC method was set up with the aim of following the course of the methylation reaction of uracil and its 5-substituted derivatives to give monomethyl and dimethyl derivatives and that of their N^1- and N^3-methyl derivatives to give $N^{1,3}$-dimethyl derivatives under different experimental conditions.

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EXPERIMENTAL

Materials

Compounds **I, III, IV, V, IX, XIII, XIV, XVII** and **XXI** were purchased from Sigma (St. Louis, MO, USA) and used after crystallization.

Silica gel 60 F_{254} TLC plates (20 × 20 cm, 0.25 mm) and silica gel 60 F_{254} HPTLC plates (10 × 20 cm, 0.20 mm) were supplied by Merck (Darmstadt, Germany).

All solvents were of analytical-reagent grade and were obtained from Merck.

Apparatus

Droplet countercurrent chromatography (DCCC). The separations were achieved on a Buchi (Flawil, Switzerland) DCCC 670 apparatus equipped with 200 columns (2.7 mm I.D.). The solvent system for all separations was chloroform–methanol–water (5:5:3, v/v/v) in the descending mode. A Buchi Model 683 UV detector with a 254-

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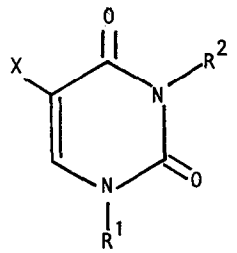
TABLE I

STRUCTURES OF URACIL COMPOUNDS



Compound	X	R^1	R^2	Compound	X	R^1	R^2
I	H	H	H	**XIII**	Br	H	H
II	H	CH_3	H	**XIV**	Br	CH_3	H
III	H	H	CH_3	**XV**	Br	H	CH_3
IV	H	CH_3	CH_3	**XVI**	Br	CH_3	CH_3
V	F	H	H	**XVII**	CH_3	H	H
VI	F	CH_3	H	**XVIII**	CH_3	CH_3	H
VII	F	H	CH_3	**XIX**	CH_3	H	CH_3
VIII	F	CH_3	CH_3	**XX**	CH_3	CH_3	CH_3
IX	Cl	H	H	**XXI**	NO_2	H	H
X	Cl	CH_3	H	**XXII**	NO_2	CH_3	H
XI	Cl	H	CH_3	**XXIII**	NO_2	H	CH_3
XII	Cl	CH_3	CH_3	**XXIV**	NO_2	CH_3	CH_3

nm filter, coupled with an LKB (Bromma, Sweden) Model 2210 recorder and an LKB Multirac 2111 fraction collector, was connected to the DCCC apparatus.

Medium-pressure liquid chromatography (MPLC). A Buchi Model 685 MPLC glass column (460 × 36 mm I.D.) was dry-filled with silica gel 60 (particle size 0.015–0.040 mm) (Merck) and connected to an LKB Multirac 2111 fraction collector. The fractions were monitored using 5 × 10 cm TLC plates.

The mobile phases for preparative MPLC separation were toluene–acetone (1:1, v/v) (system 2, Table II) for the separation of methyl derivatives of **I**, **XVII** and **XXI** and dichloromethane–ethylacetate (1:1, v/v) (system 5, Table II) for methyl derivatives of **V**, **IX** and **XIII**.

High-performance thin-layer chromatography (HPTLC). Samples were applied by means of a Linomat IV spotter (Camag, Muttenz, Switzerland) on 10 × 20 cm HPTLC plates. The solvent systems were system 2 for methyl derivatives of **I** and **XVII**, system 5 for methyl derivatives of **V**, **IX** and **XIII** and benzene–acetone (1:1, v/v) (system 1, Table II) for methyl derivatives of **XXI**.

All the plates were developed at room temperature using the ascending mode in a chromatographic tank previously saturated with eluent mixture. The layers were analysed at 254 nm by the fluorescence quenching method using a Camag TLC Scanner II linked to an Olivetti M280 PC operating the "Cats 3.04" (Camag) scanning program. The scanner was set up as follows: band with, 10 nm; span, 25; slit, 5 × 0.2 mm; and scanning speed, 5 mm/s.

Melting points were determined on a Buchi Model 510 apparatus and are uncorrected.

The compounds were analysed for C, H, N; the values obtained were within ± 0.3% of the theoretical values.

UV spectra were obtained with a Lambda 5 spectrophotometer (Perkin-Elmer, Norwalk, CT, USA) in 10^{-5} *M* buffered solution at pH 4 for the neutral form and at pH 13 or 11 for the monodeprotonated form: compounds **I**, **V**, **IX** and **XIII** are completely monodissociated at pH 11, whereas **XVII** and the N^1-methyl- and N^3-methyluracil derivatives **II**, **III**, **VI**, **VII**, **X**, **XI**, **XIV**, **XV**, **XVIII**, **XIX**, **XXII** and **XXIII** are completely dissociated at pH 13 [1,2].

Synthesis

Methylation of **I**, **V**, **IX**, **XIII**, **XVII** and **XXI** was carried out by suspending the compounds in acetonitrile and then adding tetrabutylammonium hydroxide (TBAH) (25% in methanol) (1–1.5 mol) and methyl iodide (1–1.5 mol). The mixture was stirred for 1 h at 40°C, thermostated at 40°C for 2 h and dried under vacuum; the crude material was then extracted with chloroform. The chloroform extract, consisting of starting product and N^1-methyl, N^3-methyl and $N^{1,3}$-dimethyl derivatives, was separated by DCCC and/or MPLC.

The melting points of the separated compounds were identical to those reported in the literature {**II**, **III**, **XV**, **XXII**, **XXIII** and **XXIV** [3]; **VI** [4]; **VII** [5]; **VIII** [6]; **X** [7]; **XII** [8]; **XVI**, **XVIII**, **XIX** and **XX**, [1]; **XI**, m.p. 189–191°C [acetone–light petroleum (b.p. 60–80°C] [9]}.

The physico-chemical properties of **I**–**XXIV** correspond to those reported in the literature [1,2,8].

Study of methylation reaction of uracil and 5-substituted uracil

TBAH (25% in methanol) (1 equiv.) was added to 0.2–0.3 mmol of **I**, **V**, **IX**, **XIII**, **XVII** and **XXI** in a 10-ml volumetric flask which was then filled to the mark with acetonitrile. The stirred solution was heated at 40°C and methyl iodide (1 equiv.) was added. Suitable volumes of standard solutions of starting product and of the respective methyl derivatives in acetonitrile were spotted alternately on to HPTLC plates with 2 μl of reaction mixture taken after 0, 3, 6, 10, 15, 30, 60 and 90 min.

The identities of the compounds were determined by means of the R_F values and by a computerized identity check procedure of UV spectra, which confirmed the correlation of the sample spectra with the standard spectrum. Calibration graphs were plotted for each plate by using the linear regression equation obtained from the area values under the peaks for different amounts of standard solution. The linearity correlation coefficient was between 0.997 and 0.998 for all compounds. The reproducibility of method was assessed from repeated analyses of spots containing 50 ng of compounds per spot; the relative standard deviations were between 1.8% and 3.5%. The recoveries of the compounds from artificial reaction mixtures were between 90.8% and 102.5%.

The study of the methylation reaction of N^1-methyl (**II**, **VI**, **X**, **XIV**, **XVIII** and **XXII**) and N^3-methyl derivatives (**III**, **VII**, **XI**, **XV**, **XIX** and **XXIII**) to the corresponding $N^{1,3}$-dimethyl derivatives was carried out following the procedure described above. Reactivity to the methylation of **I** was also determined at 0 and 25°C.

RESULTS AND DISCUSSION

DCCC and MPLC proved to be suitable techniques for separating the following reaction mixtures: **I**–**IV**, **V**–**VIII**, **IX**–**XII**, **XIII**–**XVI**, **XVII**–**XX** and **XXI**–**XXIV**.

In the case of the mixtures **I**–**IV**, **V**–**VIII**, **IX**–**XII**, **XIII**–**XVI** and **XVII**–**XX**, TLC analysis (Table II) revealed decreasing R_F values in the order $N^{1,3}$-di-

TABLE II

R_F VALUES OF URACIL DERIVATIVES

Compound	Solvent system[a]					
	1	2	3	4	5	6
I	0.25	0.16	0.09	0.21	0.07	0.53
II	0.33	0.23	0.10	0.21	0.12	0.69
III	0.43	0.34	0.14	0.32	0.18	0.73
IV	0.52	0.43	0.20	0.32	0.25	0.85
V	0.41	0.31	0.11	0.40	0.18	0.51
VI	0.52	0.43	0.20	0.43	0.27	0.62
VII	0.59	0.48	0.27	0.56	0.36	0.70
VIII	0.69	0.59	0.41	0.57	0.50	0.85
IX	0.45	0.39	0.21	0.51	0.28	0.63
X	0.58	0.52	0.31	0.54	0.40	0.75
XI	0.63	0.56	0.37	0.65	0.49	0.77
XII	0.76	0.67	0.51	0.67	0.61	0.91
XIII	0.45	0.39	0.17	0.54	0.29	0.55
XIV	0.60	0.52	0.32	0.58	0.44	0.70
XV	0.65	0.59	0.39	0.67	0.55	0.73
XVI	0.75	0.69	0.53	0.69	0.69	0.83
XVII	0.26	0.23	0.06	0.12	0.06	0.44
XVIII	0.42	0.37	0.12	0.20	0.15	0.61
XIX	0.53	0.48	0.22	0.35	0.22	0.62
XX	0.66	0.59	0.32	0.38	0.35	0.82
XXI	0.31	0.20	0.08	0.13	0.08	0.12
XXII	0.62	0.52	0.25	0.52	0.38	0.45
XXIII	0.55	0.47	0.25	0.38	0.30	0.41
XXIV	0.77	0.65	0.46	0.62	0.57	0.74

[a] 1 = Benzene–acetone (1:1, v/v); 2 = toluene–acetone (1:1, v/v); 3 = chloroform–ethylacetate (1:1, v/v); 4 = chloroform–ethylacetate (1:9, v/v); 5 = dichloromethane–ethylacetate (1:1, v/v); 6 = chloroform–methanol–water (5:5:3, v/v/v).

TABLE III

UV λ_{max} VALUES

Compound	Conditions[a]		Compound	Conditions[a]	
	1	2		1	2
I	259	261/262	**XIII**	276	283
II	267	272/271	**XIV**	283	289
III	259	261	**XV**	275	282
IV	266	270	**XVI**	283	288
V	266	272	**XVII**	265	269
VI	273	279	**XVIII**	273	277
VII	266	272	**XIX**	265	269
VIII	273	279	**XX**	272	276
IX	274	281	**XXI**	300	339
X	279	288	**XXII**	309	310
XI	274	282	**XXIII**	299	339
XII	280	287	**XXIV**	309	308

[a] 1 = In buffered solution at pH 4; 2 = on HPTLC plate, scanning in the reflectance mode.

TABLE IV

AMOUNTS OF $N^{1,3}$-DIMETHYL DERIVATIVES OBTAINED FROM 1 mmol OF N^1-METHYL- OF N^3-METHYL-5-URACIL DERIVATIVES AT 40°C AFTER 15 min

Starting compound	$N^{1,3}$-Dimethyl-5-uracil derivative	Amount obtained (mmol)
II	**IV**	0.83
VI	**VIII**	0.90
X	**XII**	0.88
XIV	**XVI**	0.92
XVIII	**XX**	0.98
XXII	**XXIV**	0.88
III	**IV**	0.89
VII	**VIII**	0.99
XV	**XVI**	0.69
XIX	**XX**	0.95
XXIII	**XXIV**	0.30

methyl- > N^3-methyl- > N^1-methyl > uracil, which correlated with the pK_a values [1,2].

The UV adsorption band used to identify the compounds was the higher wavelength band corresponding to the π–π^* transition of the chromophore N_1–C_6=C_5–C_4=O.

The pattern of the UV spectra of each compound was the same, whether in solution [1,2] or on the plate; in the latter instance, and with the exception of the pair **XXII**–**XXIV**, the λ_{max} was slightly higher (Table III). This difference may be attributed to a greater interaction between the substance and the chromatographic support with a consequent reduction in the π–π^* electronic transition energies, due to a lowering of π^*.

The reactions showed that, after 15 min at 40°C, methylation of the N^1-methyl and N^3-methyl derivatives with exception of the N^3-methyl-5-nitrouracil (**XXIII**) (table IV) was virtually complete and was independent of the nature of the substituent at the 5-position, and methylation of **I**, **V**, **IX**, **XIII** and **XVII** was rapid whereas that of **XXI** was slower (Table V). In every instance, mixtures of N^1-methyl and $N^{1,3}$-dimethyl derivative were formed together with small amounts of the substituted N^3-isomer and starting product.

TABLE V

AMOUNTS OF N^1-METHYL, N^3-METHYL AND $N^{1,3}$-DIMETHYL DERIVATIVES OBTAINED FROM 1 mmol OF 5-URACIL DERIVATIVES AT 40°C AFTER 15 min

Starting compound	N^1-Methyl-	Amount obtained (mmol)	N^3-Methyl-	Amount obtained (mmol)	$N^{1,3}$-Dimethyl-	Amount obtained (mmol)
I	**II**	0.56	**III**	0.01	**IV**	0.30
V	**VI**	0.33	**VII**	0.06	**VIII**	0.31
IX	**X**	0.43	**XI**	0.04	**XII**	0.23
XIII	**XIV**	0.46	**XV**	0.05	**XVI**	0.26
XVII	**XVIII**	0.29	**XIX**	0.07	**XX**	0.36
XXI	**XXII**	0.28	**XXIII**	0.03	**XXIV**	0.06

TABLE VI

AMOUNTS OF N^1-METHYL (II), N^3-METHYL (III) and $N^{1,3}$-DIMETHYL (IV) DERIVATIVES OBTAINED FROM 1 mmol OF 5-URACIL (I) AT 0, 15 and 40°C AFTER 15 min

Temperature (°C)	Amount obtained (mmol)		
	II	III	IV
0	0.18	0.30	0.15
15	0.44	0.04	0.26
40	0.56	0.01	0.30

Tests conducted on I at 0 and 25°C (Table VI) showed that as the temperature increased so did the yield of N^1- and $N^{1,3}$-dimethyl derivatives, whereas the percentage of N^3-methyl derivative remained almost constant.

CONCLUSIONS

The HPTLC apparatus gave rapid and accurate information about the reactivity of the uracil derivatives, the amount of compounds obtained and the time required to complete the reactions. In particular, it emerged that, with the exception of 5-nitrouracil, methylation, which hitherto has been continued for longer periods [1], is complete within the first 15 min. The HPTLC method allowed the reactivity of various uracil derivatives to be followed and could therefore be a helpful technique in optimizing organic reactions.

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