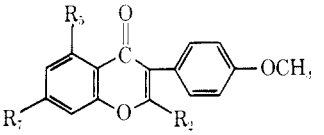
TABLE I

FORMONONETIN AND BIOCHANIN A DERIVATIVES



Compd	R_5	R_6	R_7	Method	Mp, °C	Recrystn solvent[a]	Formula	%C Calcd	%C Found	%H Calcd	%H Found	%N Calcd	%N Found	%other Calcd	%other Found
I	H	H	$OCH_2CH_2N(CH_3)_2$	A	139–141	M	$C_{20}H_{23}NO_4$	70.78	70.26	6.24	6.51	4.13	4.12		
II	H	H	$OCH_2CH_2N(C_2H_5)_2$	A, B	102.5–104	PE	$C_{22}H_{27}NO_4$	71.91	71.64	6.86	6.82	3.81	3.63		
	Hydrochloride				188–190	A								Cl: 8.79	8.58
III	CH_3	H	$OCH_2CH_2N(C_2H_5)_2$	A		PE	$C_{23}H_{29}NO_4$	72.41	72.85	7.13	7.05	3.67	3.73		
	Hydrochloride				219–221	A–E								Cl: 8.48	8.51
IV	H	OH	$OCH_2CH_2N(CH_3)_2$	A	119–120	PE	$C_{20}H_{23}NO_5$	67.59	68.04	5.96	6.14	3.92	3.91		
V	H	OH	$OCH_2CH_2N(C_2H_5)_2$	A, B	78–80	PE	$C_{22}H_{27}NO_5$	68.91	69.03	6.57	6.43	3.65	3.65		
	Hydrogen sulfate				180–182	M						2.91	2.95	S: 6.65	6.75
VI	H	OH	OCH_2CH_2N	A	80–81	PE	$C_{22}H_{25}NO_5$					3.54	3.41		
VII	H	OH	$OCH_2CH_2CH_2N(CH_3)_2$	A	90–91	PE	$C_{21}H_{23}NO_5$					3.79	3.74		
VIII	CH_3	OH	$OCH_2CH_2N(CH_3)_2$	A	115–117	PE	$C_{22}H_{25}NO_5$	68.28	68.18	6.28	6.42	3.79	3.69		
IX	CH_3	OH	$OCH_2CH_2N(C_2H_5)_2$	A	71.5–72.5	PE	$C_{23}H_{27}NO_5$	69.50	69.47	6.85	6.90	3.52	3.56		
	Hydrochloride				239–242	M–E								Cl: 8.17	8.31
X	H	H	OCH_2CH_2Br	B	174–175	A	$C_{18}H_{15}BrO_4$							Br: 21.35	20.80
XI	H	OH	OCH_2CH_2Br	B	163–165	B	$C_{18}H_{15}BrO_5$	55.26	56.02	3.86	4.06			Br: 20.43	20.70
XII	H	H	$OCH_2COOC_2H_5$	C	142–144	EA	$C_{20}H_{18}O_6$	67.79	67.45	5.12	5.12				
XIII	H	H	OCH_2COOH		223–225	DMF–M	$C_{18}H_{14}O_6$	66.26	66.50	4.32	4.51				
XIV	CH_3	H	$OCH_2COOC_2H_5$	C	111–113	B–PE	$C_{21}H_{20}O_6$	68.47	68.43	5.47	5.31				
XV	H	OH	$OCH_2COOC_2H_5$	C	160–163	EA	$C_{20}H_{18}O_7$	64.86	64.52	4.90	4.97				

[a] A = ethanol, B = benzene, E = ether, EA = ethyl acetate, PE = petroleum ether (bp 80–120°), M = methanol.

isoflavones and estrogens (such as estradiol and diethylstilbestrol)[3] as well as between isoflavones and bioflavonoids have prompted us to synthesize a series of derivatives of two natural isoflavones, biochanin A (5,7-dihydroxy-4-methoxyisoflavone) and formononetin (7-hydroxy-4′-methoxyisoflavone), different in estrogenic potency.[2] The new compounds are shown in Table I.

Experimental Section[4]

Biochanin A and formononetin were prepared by the Baker method.[5] The final step (decarboxylation of the corresponding 2-carboxylic acids) was accomplished, for both compounds, by heating under nitrogen at 300° for 15 min and subliming the crude products at 10^{-3} mm; yields, 88–90%. 2-Methylbiochanin A and 2-methylformononetin were synthesized by known methods.[6]

7-Diethylaminoethoxy-5-hydroxy-4′-methoxyisoflavone (V). Method A.—To a stirred suspension of 5.68 g (0.02 mole) of biochanin A in 40 ml of anhydrous methanol was added 0.02 mole of $NaOCH_3$ (9.2 ml of 11.75% solution in methanol). After a few minutes, 60 ml of xylene was added and methanol was distilled completely under reduced pressure. Diethylaminoethyl chloride (4 g, 0.02 mole) was added, and the mixture was heated in an oil bath at 110° for 2 hr, then filtered and extracted with 10% acetic acid. The acid extract was filtered and rendered slightly alkaline with NH_4OH. The precipitate was collected, dried, and recrystallized from petroleum ether (bp 80–120°) giving 6.7 g (88%) of V, mp 78–80°. The ultraviolet spectrum (λ_{max} 260 mμ in ethanol and in 4% ethanolic sodium acetate, 272 mμ in 4% ethanolic $AlCl_3 \cdot 6H_2O$) was consistent with a free 5-hydroxyl group.[7] The infrared spectrum, similarly, did not show absorption in 3500–3300-cm^{-1} region.[8] The hydrogen sulfate, precipitated by adding concentrated H_2SO_4 to an acetone solution of V and recrystallized from methanol had mp 180–182°.

Method B.—To a solution of 5.68 g (0.02 mole) of biochanin A in 130 ml of anhydrous Cellosolve was added 0.02 mole of $NaOCH_3$ (9.2 ml of 11.75% solution in methanol). The mixture was distilled until the boiling point of Cellosolve was reached, then 30 ml of dibromoethane was added, and the mixture was refluxed for 2 hr and evaporated to dryness under reduced pressure. The residue was triturated with 5% NaOH, washed thoroughly with water, dried, and recrystallized from benzene giving 5.5 g (70%) of 7-bromoethoxy-5-hydroxy-4′-methoxyisoflavone (XI), mp 163–165°. A solution of 3.91 g of XI in 35 ml of diethylamine and 35 ml of dimethylformamide was kept for 4 days at room temperature, then evaporated to dryness under reduced pressure. The residue, triturated with water, dried, and recrystallized from petroleum ether gave V, mp 78–80°, identical with the product obtained by method A.

Ethyl 4-Methoxy-7-isoflavonoxyacetate (XII). Method C.—To a stirred suspension of 0.02 mole of formononetin sodium salt in xylene, prepared as in method A, was added 10.6 g (0.06 mole) of ethyl bromoacetate. The mixture was refluxed for 5 hr, filtered, and evaporated under reduced pressure. The residue was triturated with petroleum ether. Recrystallization from ethyl acetate or benzene yielded 5.9 g (83%) of XII, mp 142–144°.

4′-Methoxy-7-isoflavonoxyacetic Acid (XIII).—A mixture of 2 g of XII, 30 ml of acetone, 4 ml of water, and 3.25 ml of 2 *N* NaOH was stirred for 2 hr. Water was added and acetone was evaporated under reduced pressure. On acidification of the clear solution with HCl, 1.45 g (79%) of XIII, mp 223–225°, was precipitated.

(3) C. A. B. Clemetson, L. Blair, and A. B. Brown, *Ann. N. Y. Acad. Sci.*, **93**, 279 (1962).

(4) Melting points were taken in capillaries and are corrected.

(5) W. Baker, J. Chadderton, J. B. Harborne, and W. D. Ollis, *J. Chem. Soc.*, 1852 (1953).

(6) (a) W. Baker and R. Robinson, *ibid.*, 2713 (1926); (b) V. N. Gupta and T. R. Seshadri, *J. Sci. Ind. Res.* (India), **16B**, 116 (1957); *Chem. Abstr.*, **52**, 9007 (1958).

(7) R. M. Horowitz and L. Jurd, *J. Org. Chem.*, **26**, 2446 (1961).

(8) A. Jacot-Guillarmod and A. Pigueı, *Helv. Chim. Acta*, **46**, 49 (1963).

Octamethylbiguanide Perchlorate

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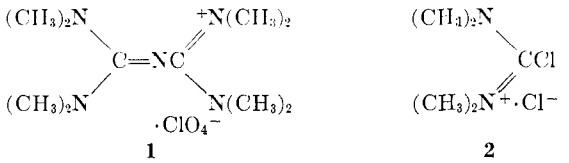
Although large numbers of biguanides have been synthesized as potential antimalarial drugs[1] and as hypoglycemic agents,[2] the number of polysubstituted examples is limited. In this paper, we describe the synthesis of octamethylbiguanide perchlorate (**1**), the most highly substituted biguanide yet reported.

The reaction of tetramethylchloroformamidine chloride (**2**) and 1,1,3,3-tetramethylguanidine provided a hygroscopic chloride

(1) F. H. S. Curd and F. L. Rose, *J. Chem. Soc.*, 729 (1946).

(2) S. L. Shapiro, V. A. Parrino, and L. Freedman, *J. Am. Chem. Soc.*, **81**, 3728 (1959).



1 2

salt which was converted to the easily purified perchlorate **1**. The identity of the product was confirmed by analytical and spectral data.

Experimental Section[3]

Tetramethylchloroformamidine Chloride (**2**).—To a stirred solution of 54.0 g (0.47 mole) of tetramethylurea in 1.2 l. of anhydrous ether was added dropwise 60.0 g (0.47 mole) of oxalyl chloride. After 2 days, the mixture was filtered, and the solid was washed with ether and dried. The product amounted to 69.0 g (87%) of colorless crystals, mp 145–150° dec, of purity suitable for further use. Four recrystallizations of a small portion from anhydrous acetonitrile provided extremely hygroscopic colorless prisms, mp 150–155° dec (lit.[4] mp 110–112°).

Anal. Calcd for $C_5H_{12}Cl_2N_2$: C, 35.10; H, 7.07; Cl, 41.44; N, 16.37. Found: C, 34.56; H, 7.02; Cl, 41.03; N, 16.00.

Octamethylbiguanide Perchlorate (**1**).—To a stirred suspension of 15.0 g (0.088 mole) of tetramethylchloroformamidine chloride in 50 ml of anhydrous acetonitrile was added dropwise 25 ml of 1,1,3,3-tetramethylguanidine. The solid precipitate, which was collected after 2 days, was tetramethylguanidine hydrochloride, mp 204–206°. The filtrate was diluted with 400 ml of ether, and an oil separated, which on standing formed 22 g of extremely hygroscopic colorless crystals, mp 115–150°. The solid was stirred for 2 hr with a suspension of 47 g of freshly prepared Ag_2O in 250 ml of ethanol and 5 ml of water. The mixture was filtered, and the filtrate was adjusted to pH 7 with 11 ml of 70% perchloric acid. The solid which separated amounted to 26 g (94%) of colorless crystals, mp 192–199°. Four recrystallizations from ethanol provided the analytical sample, colorless prisms, mp 197–198°.

Anal. Calcd for $C_{10}H_{24}ClN_5O_4$: C, 38.27; H, 7.70; Cl, 11.30; N, 22.32. Found: C, 38.46; H, 7.77; Cl, 11.05; N, 22.36.

The ultraviolet spectrum exhibits in methanol a maximum at 243 mμ (ε 31,000), which shifts to 226 mμ (ε 24,800) in 0.1 *N* methanolic HCl.[5] The nmr spectrum in DMSO-d_6 exhibits a sharp singlet τ 7.20.

(3) Melting points were determined in a Hershberg apparatus and are uncorrected. Ultraviolet spectra were determined with a Cary 11 spectrophotometer and nmr spectra were determined with a Varian A-60 spectrometer by Mr. W. Fulmor and staff. Microanalyses were performed by Mr. L. M. Brancone and staff.

(4) H. Eilingsfeld, M. Seefelder, and H. Weidinger, *Angew. Chem.*, **72**, 836 (1960); H. Eilingsfeld, G. Nebauer, M. Seefelder, and H. Weidinger, *Chem. Ber.*, **97**, 1232 (1964).

(5) The correlation between the ultraviolet spectrum and tautomeric structure of biguanides has been discussed: W. J. Fanshawe, V. J. Baller, E. F. Ullman, and S. R. Safir, *J. Org. Chem.*, **29**, 308 (1964).

6-Trimethylammoniopurinide[1]

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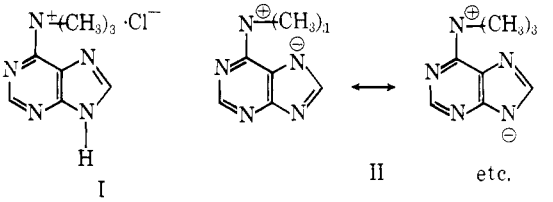
Current clinical interest[2] in the carcinostatic activity of the antimetabolite 6-purinyltrimethylammonium chloride[3] (I) led us to investigate methods for the purification of this salt. Precipitation of its aqueous solution by organic solvents consistently yielded products low in chlorine.[4] During further study, an aqueous solution of I was passed through an anion-exchange column in its hydroxide form, yielding on evaporation a new substance, mp 190–192°, which was chlorine free and had structure II (see Experimental Section).



I II etc.

Experimental Section

Purin-6-yltrimethylammonium chloride (I) was prepared by the method of Horwitz and Vaitkevicius.[3] Small quantities of crude I were purified by solution in cold water and quick precipitation with acetone; pure I melts at 191–192°.

6-Trimethylammoniopurinide (II).—A solution of 170 g of I in 1 l. of water was passed through an ion-exchange column with 1.5 l. of Dowex 1-X8 (hydroxyl form), and the column was then washed thoroughly with water. The combined eluates were then evaporated to dryness *in vacuo* at 50°, and the product crystallized from water and was dried *in vacuo* (P_2O_5) to yield 112 g of II, mp 190–192°.

Anal. Calcd for $C_8H_{11}N_5$: C, 54.22; H, 6.26; N, 39.52. Found: C, 54.30, 54.30; H, 6.03, 6.20; N, 39.18, 39.49.

Structure II is supported by the nmr spectrum[5] in D_2O which shows, besides two equal peaks at 221 and 212 cycles below the signal from solvent protons, only one, much more intense, peak at 59 cycles above solvent reference. The dipolar salt II has the same melting point as 6-dimethylamino-1-methylpurine[6] (III), and the melting points of the respective picrates are also similar. However, the mixture melting point of II and III is depressed, the maximum of the ultraviolet spectrum of II is at much lower wavelength than that of III, and the nmr spectrum of III shows two well-separated methyl group signals. Electrometric titration shows that II is a weak base and the pK_a of the conjugate acid I is 6.8. This value appears reasonable, for purine has $pK_a = 8.9$,[7] and the trimethylammonium group would lower the pK.[8]

Similarly, the ultraviolet spectrum of II appears reasonable [$\lambda_{max}^{H_2O}$ (pH 10) 274 mμ (log ε 3.86)] when compared with that of purine anion[9] [λ_{max} (pH 11) 271 mμ (log ε 3.88)]; the trimethylammonium group, being nonconjugating, would be expected to have little effect. The spectrum of II at pH 1 (that is, of I), with λ_{max} 265 mμ (log ε 3.94), is also close to that of neutral purine [λ_{max} 263 mμ (log ε 3.90)].

Saline solutions of I and II are indistinguishable.

(1) Alpurine (Trademark applied for).

(2) V. K. Vaitkevicius and M. L. Reed, *Proc. Am. Assoc. Cancer Res.*, **7**, 72 (1966).

(3) J. P. Horwitz and V. K. Vaitkevicius, *Experientia*, **17**, 552 (1961).

(4) Similar material, which can now be construed as a solvated equimolar mixture of I and II, was obtained by E. J. Reist, A. Benitez, L. Goodman, B. R. Baker, and W. W. Lee, *J. Org. Chem.*, **27**, 3274 (1962).

(5) We wish to thank Dr. George Slomp for determining the nmr spectrum of II.

(6) (a) L. B. Townsend, R. K. Robins, R. N. Loeppky, and N. J. Leonard, *J. Am. Chem. Soc.*, **86**, 5320 (1964); (b) B. C. Pal and C. A. Horton, *J. Chem. Soc.*, 400 (1964); (c) we wish to thank Professor R. K. Robins for a sample of III.

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(8) H. C. Brown, D. H. McDaniel, and O. Häflinger in "Determination of Organic Structures by Physical Methods," E. A. Braude and F. C. Nachod, Ed., Academic Press Inc., New York, N. Y., 1955, p 592.

(9) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).