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POTENTIAL ANTICANCER COMPOUNDS. III. SYNTHESIS OF SOME 8-SUBSTITUTED CAFFEINES AND THEOPHYLLINES

HANS ZIMMER, JOSEPH B. METTALIA, JR., AND R. ATCHLEY

Department of Chemistry, University of Cincinnati, Cincinnati 21, Ohio

The concept of the use of antimetabolites as a possible route to a successful cancer chemotherapy has prompted the synthesis of numerous potential purine antagonists (Usbeck et al., 1961). The majority of these compounds were derived from purine, xanthine, adenine or hypoxanthine. Caffeine and theophylline, however, have obtained little attention as purine components in potential purine antagonists. Therefore, it was decided to synthesize a number of 8-substituted caffeines for testing against experimental cancer in mice. 8-N-nitroso-N-alkylaminocaffeines were prepared for reasons outlined in an earlier paper (Zimmer and Swamy, 1959); the 8-N-mustard derivatives were included in this study because of the concept (Arnold et al., 1958; Bergel, 1958) that active alkylating agents are composed of a carrier moiety and an alkylating function.

The 8-alkylaminocaffeines were prepared by reacting 8-bromocaffeine with the appropriate amine in refluxing butanol. The results are shown in table 1. A few alkylated 8-aminocaffeines have been reported (Fischer, 1882; Cramer, 1894; Einhorn and Baumeister, 1898; Blicke and Godt, 1954). They were prepared by reacting 8-bromocaffeine and the appropriate amine in a solvent under pressure. The methods used in this study avoid the inconvenient closed tube reactions. The yields obtained compare favorably, however, with the latter method. Attempts to prepare 8-*tert*-butylaminocaffeine by this method as well as by reacting 8-bromocaffeine and *tert*-butylamine in the presence of sodium amide in refluxing toluene were unsuccessful. The reaction of 8-bromocaffeine with amines appeared to be controlled to some extent by a steric factor. This is borne out by the facts that, (1) no tertiary amines were produced in the reaction, (2) yields decreased with increased branching of the α -carbon atom of the amine, and (3) morpholine, a strong basic amine in which the alkyl groups are "tied back," gave good yields. To further confirm the steric nature of this effect the reaction 8-chloromethylcaffeine with *tert*-butylamine was examined. 8-*tert*-butylaminomethylcaffeine should be relatively free from steric hindrance between the imidazole ring of caffeine and the alkyl group of the amine. As anticipated 8-*tert*-butylaminomethylcaffeine was obtained.

The nitrosation of these amines was accomplished by two methods; namely, by using nitrous acid and nitrosyl chloride (Newman and Kutner, 1951) as nitrosating agents. The results are shown in table 2. N-Nitroso derivatives of caffeines and theophyllines have only been recently reported (Zelnik et al., 1956). Low temperature recrystallization and storage under a low pressure of nitrogen were employed to minimize the decomposition of the prepared compounds. This instability may be due possibly to the fact that these N-nitrosamines are structurally comparable to N-nitrosamides which are known to be unstable and to undergo rearrangements (Bamberger, 1897; Huisgen and Reimlinger, 1956; Hey et al., 1952; White, 1955).

Attempts to synthesize N-(8-caffeinyl)glycine, as a potential amino acid analog, by different methods were unsuccessful. One of the methods planned was to proceed according to the following reaction sequence:

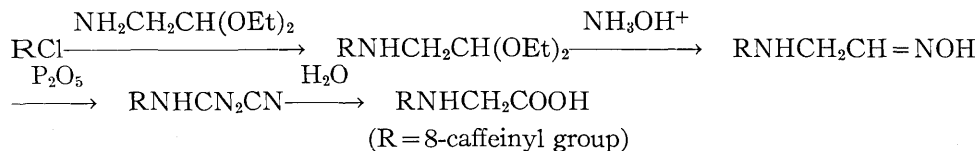
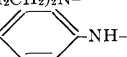


TABLE 1
Mono- and disubstituted 8-aminocaffeines

No. Substituent(s)	%	Yield	M.P.	Empirical formula	Analysis					
					Calculated			Found		
					C	H	N	C	H	N
I n-C ₃ H ₇ NH-	82 ^a		238-240	C ₁₁ H ₁₇ N ₅ O ₂	52.6	6.8	—	52.3	6.7	—
II n-C ₄ H ₉ NH-	85		225-227	C ₁₂ H ₁₉ N ₅ O ₂	54.3	7.2	—	54.2	7.6	—
III n-C ₆ H ₁₃ NH-	75		195-196	C ₁₄ H ₂₃ N ₅ O ₂	57.3	7.9	—	57.2	7.9	—
IV i-C ₃ H ₇ NH-	57		241-243	C ₁₁ H ₁₇ N ₅ O ₂	52.6	6.8	—	52.6	6.9	—
V sec-C ₄ H ₉ NH-	27		216-218	C ₁₂ H ₁₉ N ₅ O ₂	54.3	7.2	—	54.4	7.3	—
VI O(CH ₂ CH ₂) ₂ N-	74 ^b		166	C ₁₂ H ₁₇ N ₅ O ₂	51.6	6.1	—	51.4	6.2	—
VII H ₂ N-  -NH-	0.5		>320	C ₁₄ H ₁₆ N ₅ O ₂	56.0	5.4	—	55.4	5.3	—
VIII -HNCH ₂ CH ₂ NH-	67		>320	C ₁₈ H ₂₄ N ₁₀ O ₄	48.6	5.4	—	48.4	5.3	—
IX HOCH ₂ CHNH-	90		232-234	C ₁₀ H ₁₅ N ₅ O ₃	47.4	6.0	—	49.3	6.1	—
X (HOCH ₂ CH ₂) ₂ N-e	44		138-139 ^d	C ₁₂ H ₁₉ N ₅ O ₄	48.5	6.4	23.6	48.8	6.6	23.5
XI (ClCH ₂ CH ₂) ₂ N ^e	52		151-153(dec)	C ₁₂ H ₁₇ Cl ₂ N ₅ O ₄ ^e	43.1	5.1	21.0	43.6	5.1	20.2

^aYields are of recrystallized compound.

^bBlicke and Godt (1954); a yield of 66 per cent was reported, m.p. 166-167°.

^cThe corresponding theophylline derivatives were analogously prepared: 8-Bis(2-hydroxyethyl)aminotheophylline obtained in 64 per cent yield, m.p. 246-248°; Anal. Calcd. for C₁₁H₁₇N₅O₄: C, 46.6; H, 6.1; N, 24.7. Found: C, 46.5; H, 6.2; N, 24.2; 8-bis(2-chloroethyl)aminotheophylline obtained in 57 per cent yield, m.p. 177°, it resolidifies and exhibits a second m.p. 205-208°. There is no OH-peak in the IR-spectrum. Anal. Calcd. for C₁₁H₁₅Cl₂N₅O₂: C, 41.3; H, 4.7; Cl, 22.2; N, 21.9. Found: C, 41.9; H, 4.7; Cl, 22.1; N, 20.9.

^dIt also showed resolidification after first melting at 124-126°.

^eCl-analysis Calcd.: 21.2; found: Cl, 21.3. There is no OH-peak in the IR-spectrum.

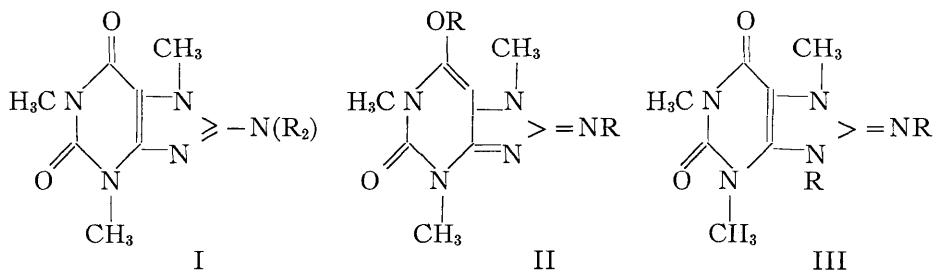
TABLE 2
8-(N-nitroso-N-alkyl)aminocaffeines

No. 8-(N-alkyl) group	Yield	M.P.	Solvent of recryst.	Empirical Formula	Analysis			
					Calculated		Found	
					C	H	C	H
XII C ₂ H ₅	52 ^a 56 ^b	106-108	^c	C ₁₀ H ₁₄ N ₆ O ₃	45.1	5.3	45.8	5.8
XIII n-C ₄ H ₉	58 ^a 47 ^b	77-79	^c	C ₁₂ H ₁₈ N ₆ O ₃	49.0	6.2	49.1	6.4
XIV n-C ₆ H ₁₃	91 ^a	53-55	^d	C ₁₄ H ₂₂ N ₆ O ₃	52.2	6.9	52.1	7.0
XV -CH ₂ CH ₂ -	58 ^a	>320	^e	C ₁₈ H ₂₂ N ₁₂ O ₆	43.0	4.4	42.5	4.9

^aHONO-method ^bNOCl-method ^cHexane ^dNone ^eEthanol-chloroform

The desired 2,2-diethoxy-N-(8-caffeinyl)ethylamine was obtained in good yield. However, this compound reacted with hydroxylamine not as anticipated. Even at a pH as weak as 4 it gave a product which was identified as 8-aminocaffeine by its elemental analysis, infrared spectrum and by its conversion to the known 8-acetamidocaffeine and 8-diacetamidocaffeine. This totally unexpected reaction was repeated several times with the same result. Further examination revealed that a reaction occurred already in very weak acidic solution to produce 8-aminocaffeine and some black tarry material. No attempts to find out the mechanism of this reaction have been undertaken.

The question of whether 8-aminocaffeine can exhibit tautomerism was of importance in determining the correct structures of some of its derivatives which were prepared to identify it. In particular, the known 8-diacetamidocaffeine ($R = \text{COCH}_3$) could conceivably have the structures:



The existence of tautomerism was indicated by the fact that 8-aminocaffeine exhibits a very sharp triplet peak in the NH and OH region of the IR- spectrum (3.0 to 3.2).

This could be explained by assuming 8-aminocaffeine ($R = \text{H}$) to exist in either or both of the following tautomeric equilibria: $\text{I} \rightleftharpoons \text{II}$ and $\text{I} \rightleftharpoons \text{III}$.

An equilibrium mixture of I and II would contain the groups $-\text{OH}$, $=\text{NH}$ and $-\text{NH}_2$ and an equilibrium mixture of I and III would contain the groups $=\text{NH}$, $-\text{NH}_2$ and $>\text{NH}$, thus accounting for a triplet in this region of the IR-spectrum. The UV- absorption spectra of 8-aminocaffeine and some related compounds were determined in an additional effort to shed more light on this question. The spectra are shown in table 3.

TABLE 3
*The ultraviolet absorption spectra of some
8-substituted caffeines in ethyl alcohol*

R	$\lambda_{\text{max.}}$ (m μ)	ϵ
H	273	8,398
NH ₂	290	14,855
NHCOCH ₃	281	12,430
N(COCH ₃) ₂	278	10,551
NHC ₄ H ₉	297	15,652

The fact that 8-aminocaffeine absorbs at a longer wavelength than caffeine indicates that this peak must be due to either I or II. The position of the absorption excludes III from being present in significant amounts in the equilibrium of the tautomers since its chromophore is shorter than the one of I or of II or of caffeine and therefore should absorb at shorter wavelengths. This interpretation

was further confirmed by the fact that the acetyl and diacetyl derivatives of 8-aminocaffeine absorb at wavelengths intermediate between the ones of caffeine and 8-aminocaffeine; furthermore, the *n*-butyl derivative absorbs as expected at a longer wavelength than 8-aminocaffeine.

These data showed that the tautomerism must involve compounds I and II and not I and III. These conclusions also agreed with the fact that 8-acetylaminocaffeine is soluble in diluted cold aqueous NaOH-solution (Farbwerke Hoechst, 1903). The data did not indicate, however, whether the acetyl derivatives are O- or N-derivatives.

8-Aminocaffeine was reacted with *p*-nitrobenzaldehyde and *p*-dimethylaminobenzaldehyde and the expected Schiff bases were obtained.

Preliminary biological test results on compounds I to IX obtained with mouse sarcoma-180, adenocarcinoma Ca-755, and leukemia L-1210 showed no activity. Of compounds X, XI and the two theophylline derivatives no test results have been obtained as yet.

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EXPERIMENTAL

All melting points are uncorrected. The IR- spectra were obtained with a Baird IR-spectrophotometer, model KM-1 and the UV- absorption spectra were obtained with a Cary recording spectrophotometer, Model No. 11. The elemental analyses were performed by A. Bernhardt, Mülheim Ruhr, Germany.

8-Alkylaminocaffeines

These compounds were prepared by refluxing a solution of 0.07 to 0.11 moles of 8-bromocaffeine with 0.3 to 0.9 moles of the appropriate amine in 150 ml of *n*-butanol for 17 hr. The solution was cooled to 0 C and the precipitate recrystallized from ethanol.

8-(N-Nitroso-N-alkyl)aminocaffeines

HONO method.—Approximately 0.018 moles of 8-alkylaminocaffeine was dissolved in 250 ml of conc. hydrochloric acid. The solution was then diluted to 1000 ml with water, cooled to 10 C and stirred vigorously while 0.02 moles of sodium nitrite [0.04 moles in the case of N,N'-di(8-caffeinyl)ethylene diamine] in 50 ml of water were added slowly over a period of 30 min. After the addition was completed a yellow precipitate formed. The mixture was stirred an additional 2 hr and then the precipitate was collected. Recrystallizations were accomplished by dissolving the N-nitrosoamines in an appropriate solvent at a temperature below 40 C. The solutions were then filtered to remove decomposed material, the filtrate cooled to -20 C and the precipitated product collected. After several recrystallizations the bright yellow samples were stored under nitrogen at a pressure of about 2 mm of mercury. These samples show no sign of decomposition after standing for 2 yr. Samples stored not under nitrogen changed to a black tar after a few months.

NOCl Method.—The method used was that of Newman and Kutner (1951). Recrystallization procedure was the same as reported above.

8-tert-Butylaminomethylcaffeine

A solution of 1 g of crude Schloro-methylcaffeine (Boehringer, C. F. and Sons, 1902) (0.004 moles) and 30 ml of *tert*-butylamine in 30 ml of *n*-butanol was refluxed for 19 hr. After cooling 0.3 g (30 per cent) of a white precipitate was collected and recrystallized from hexane, m.p. 189–191°.

Anal. Calcd. for $C_{13}H_{21}N_5O_2$: C, 55.9; H, 7.1; N, 25.1.

Found: C, 56.0; H, 7.0; N, 24.5.

2,2-Diethoxy-N-(8-caffeinyloxy)ethylamine

A solution of 23 g of 8-chlorocaffeine (0.1 moles), 20 g of 2,2-diethoxyethylamine (0.15 moles) and 10 ml of triethylamine in 250 ml of *n*-butanol was refluxed for 19 hr. The solution turned black during the refluxing. After cooling to 0 C, a brown precipitate was collected and washed with ether until it was white. Recrystallization from ethanol gave 20 g (61.5 per cent) of white crystals. After recrystallizations the m.p. was raised to 183–184° (dec.).

Anal. Calcd. for $C_{14}H_{23}N_5O_4$: C, 51.7; H, 7.1.

Found: C, 51.4; H, 7.2.

Reaction of 2,2-diethoxy-N-(8-caffeinyloxy)ethylamine with hydroxylamine

A solution of 5 g of 2,2-diethoxy-N-(8-caffeinyloxy)ethylamine (0.015 moles) and 6 g of hydroxylamine hydrochloride (0.07 moles) was buffered to a pH of 4.4 with 120 ml of an aqueous acetic acid-sodium acetate mixture and then refluxed for 16 hr. A tan precipitate started to form during the reaction. The solution was cooled and 2.5 g of material (m.p. >320°) was collected and washed with water, ethanol and ether. This product was identified as 8-aminocaffeine by (1) the characteristic triplet peak it exhibited in the $-NH_2$ region of the IR- spectrum, (2) the formation of a diazonium salt which gave a coupling product with β -naphthol, (3) conversion to the known 8-acetamidocaffeine and 8-diacetamidocaffeine and (4) elemental analysis. The yield of 8-aminocaffeine was 79 per cent.

Anal. Calcd. for $C_8H_{11}N_5O_2$: C, 45.9; H, 5.3; N, 33.5.

Found: C, 45.90; H, 5.6; N, 33.3.

Diacetyl derivative.—m.p. 143–146° (lit. m.p. 145°) (Farbwerke Hoechst, 1903).

Anal. Calcd. for $C_{12}H_{15}N_5O_4$: C, 49.1; H, 5.2; N, 23.9.

Found: C, 49.7; H, 5.2; N, 23.8.

Monoacetyl derivative.—m.p. 274–275° (dec.) (lit. m.p. 270°) (Farbwerke Hoechst, 1903).

Anal. Calcd. for $C_{10}H_{13}N_5O_4$: C, 47.8; H, 5.2; N, 27.9.

Found: C, 47.8; H, 5.6; N, 27.8.

8-(p-Nitrobenzylidene)aminocaffeine

A solution of 1 g of 8-aminocaffeine (0.005 moles) and 1.5 g of *p*-nitrobenzaldehyde (0.010 moles) in 60 ml of glacial acetic acid was refluxed for 14 hr. After cooling a yellow precipitate was collected which was recrystallized from ethanol to give 0.4 g (22 per cent) of yellow crystals, m.p. 299–301° (dec.). After three recrystallizations the m.p. was raised to 300–302° (dec.).

Anal. Calcd. for $C_{15}H_{14}N_6O_4$: C, 52.63; H, 4.12; N, 24.55.

Found: C, 53.09; H, 5.13; N, 24.32.

8-(p-Dimethylaminobenzylidene)aminocaffeine

Obtained analogously; m. p. 297–299°.

Anal. Calcd. for $C_{17}H_{20}N_6O_2$: C, 60.0; H, 5.9; N, 24.7.

Found: C, 60.0; H, 6.0; N, 24.9.

8-Bis(2-hydroxyethyl)aminotheophylline

To a suspension of 12.95 g. 8-bromotheophylline (0.05 moles) in 200 ml of refluxing cyclohexanol was slowly added 10.5 g (0.10 moles) of diethanolamine in 40 ml of cyclohexanol. The reaction mixture which became clear was kept re-

fluxing for 24 hr. On cooling a precipitate deposited, which was filtered and repeatedly recrystallized from ethanol giving 9.08 g (63.6 per cent) of product, m.p. 246–248°. (Analysis see table 1).

8-Bis(2-chloroethyl)aminotheophylline

This compound was obtained in 56.8 per cent yield from 2.3 g of the corresponding alcohol following a method described by Benitez et al. (1960). (Analysis and m.p. see table 1).

8-Bis(2-hydroxyethyl)aminocaffeine (X)

A mixture of 2.73 g (0.01 moles) of 8-bromocaffeine, 2.1 g (0.02 moles) of diethanolamine and 50 ml of ethyl cellosolve was refluxed for 16 hr. After cooling down and evaporating off most of the solvents the resulting oil was repeatedly extracted with hot benzene which was combined, treated with Norit and filtered. Upon cooling, crystals precipitated yielding 1.30 g (43.8 per cent) of X (Analysis and m.p. see table 1).

8-Bis(2-chloroethyl)aminocaffeine (XI)

This compound was obtained from 2.97 g (0.01 mole) of X exactly as described for the theophylline analog with a 1.75 g (52.4 per cent) yield. (Analysis and m.p. see table 1).

SUMMARY

The synthesis of several 8-(N-substituted)aminocaffeines as potential anti-cancer compounds are reported. 8-N-nitrocaffeines and Schiff bases derived from 8-aminocaffeine are prepared for the first time. UV- and IR- spectra of 8-aminocaffeines are discussed in terms of possible tautomerism of these compounds.

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