Notes

Some Biologically Active N^6-Methylated Adenosine Analogs

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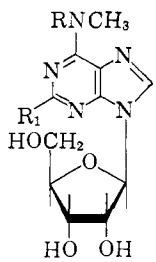
Several 2-substituted adenosines have been shown to possess vasodepressor properties of greater duration of action and of greater potency than those of adenosine,[1,2] and to inhibit the adenosine diphosphate mediated agglutination of blood platelets *in vivo* and *in vitro*.[3,4] 2-Chloroadenosine is the most potent of these analogs and would appear to be of potential therapeutic value in the treatment of hypertension or of thrombosis, except for the concomitant toxic effects it has on the mammalian heart. This analog has been shown to cause heart block in the guinea pig[2] and in man.[5]

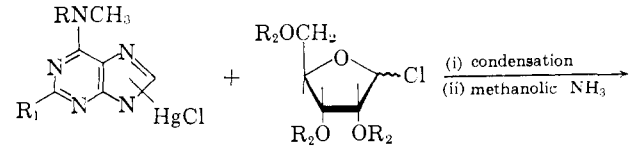
As a first approach to structural alteration which may give analogs having the beneficial vasodilatatory effects of 2-substituted adenosines without their concomitant toxic heart effects, the N^6-monomethylated derivatives of five 2-substituted adenosines, 2-chloro-, 2-methylthio-, 2-methoxy-, 2-ethylthio-, and 2-trifluoromethyladenosines (**1a–e**), were synthesized, together with 2-chloro-N^6-dimethyladenosine (**1f**).



1a, R = H; R_1 = Cl
b, R = H; R_1 = SCH_3
c, R = H; R_1 = OCH_3
d, R = H; R_1 = SCH_2CH_3
e, R = H; R_1 = CF_3
f, R = CH_3; R_1 = Cl

The analogs (**1a** and **d–f**) were prepared by Davoll's modification[6] of the classic Fischer–Helferich purine nucleoside synthesis[7] (Chart I). The appropriate 2-substituted 6-methylaminopurines were converted to their chloromercuri salts (**2**) and these were condensed with 2,3,5-tri-*O*-benzoyl-D-ribosyl chloride (**3**). Removal of the benzoyl blocking groups with methanolic ammonia gave the required nucleosides.

CHART I



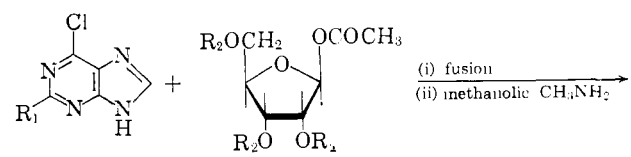
2a, R = H; R_1 = Cl
d, R = H; R_1 = SC_2H_5
e, R = H; R_1 = CF_3
f, R = CH_3; R_1 = Cl

3, R_2 = COC_6H_5

1a and **d–f**

2-Chloro-N^6-methyladenosine (**1a**) was also obtained *via* the fusion method[8] (Chart II). 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**5**) was fused with 2,6-dichloropurine (**4a**) in the presence of *p*-toluenesulfonic acid to give the blocked 2,6-dichloropurineriboside[9a] which was simultaneously deblocked and methylaminated by treatment with anhydrous methylamine in methanol at room temperature. By this method **1a** was obtained in 42% yield.

CHART II



4a, R_1 = Cl
b, R_1 = SCH_3

5, R_2 = COC_6H_5

1a and **b**

The fusion method was also used for the preparation of 2-methylthio-N^6-methyladenosine (**1b**),[10] starting with 2-methylthio-6-chloropurine (**4b**). It has been reported[8b] that fusion of **4b** with tetra-*O*-acetyl-β-D-ribofuranose in the presence of *p*-toluenesulfonic acid, followed by treatment of the blocked intermediate with methanolic ammonia gave a 36% yield of 9-β-D-ribofuranosyl-2-methylthio-6-chloropurine. In our hands fusion of **4b** with either **5** or tetra-*O*-acetyl-β-D-ribofuranose in the presence of *p*-toluenesulfonic acid, followed by prolonged treatment at room temperature of the intermediate blocked nucleoside with methanol saturated with methylamine gave only a poor yield of **1b**.

(1) D. A. Clarke, J. Davoll, F. S. Philips, and G. B. Brown, *J. Pharmacol. Exptl. Therap.*, **106**, 291 (1952).
(2) R. H. Thorp and L. B. Cobbin, *Arch. Intern. Pharmacodyn.*, **117**, 95 (1959).
(3) G. V. R. Born, A. J. Honour, and J. R. A. Mitchell, *Nature*, **202**, 761 (1964).
(4) G. V. R. Born, *ibid.*, **202**, 95 (1964).
(5) R. H. Thorp, private communication.
(6) J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951)
(7) E. Fischer and B. Helferich, *Chem. Ber.*, **47**, 210 (1914).
(8) For the acid-catalyzed fusion reaction of purines with tetra-*O*-acyl-D-ribofuranose to give β-nucleosides see (a) T. Sato, T. Simadate, and Y. Ishido, *Nippon Kagaku Zasshi*, **81**, 1440 (1960); (b) T. Simadate, Y. Ishido, and T. Sato, *ibid.*, **82**, 938 (1961); (c) G. Gough and M. H. Maguire, *J. Med. Chem.*, **8**, 866 (1965).
(9) (a) In subsequent work we found that complete reaction of 2,6-dichloropurine with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose was achieved without a catalyst simply by fusion for 45 min *in vacuo* at 130–140°. (b) Fusion of acetylated 2-methylthio-6-chloropurine with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose without a catalyst gave a clear melt at 160°, but there was no reaction. The attempted fusion of 2-methylthio-6-chloropurine with the sugar without a catalyst at 185–195° resulted only in considerable decomposition. The reactivity of other 2-substituted 6-chloropurines in the fusion reaction with and without a catalyst is presently being investigated.
(10) M. Ikehara, T. Ueda, S. Horikawa, and A. Yamazaki, *Chem. Pharm. Bull.* (Tokyo), **10**, 665 (1962), reported that reaction of $MeNH_2$ with 2,6-dimethylthio-9-(2,3,5-tri-*O*-benzoyl)-β-D-ribofuranosylpurine gave **1b**, which was isolated as a picrate salt. Spectral data only were reported: $\lambda_{max}^{0.1\ N\ HCl}$ 271 mμ, $\lambda_{max}^{0.1\ N\ NaOH}$ 279, 239 mμ.

A methylthio substituent in the 2 position of purine has base-weakening properties, thus the pK_{a1} of purine is 2.48 while that of 2-methylthiopurine is 1.89.[11] The 6-chloro group in the intermediate blocked nucleoside resulting from fusion of **4b** with **5** would therefore be expected to be almost as reactive as the 6-chloro group in blocked 2,6-dichloropurineriboside, and should be replaced by a methylamino group under similar conditions. It would appear that the poor yield of **1b** was due to incomplete reaction of **4b** with **5** in the fusion step. However, the use in this step of acetylated 2-methylthio-6-chloropurine which has a lower melting point than **4b** and fused with **5** more readily did not give an improved yield; indeed in the reaction 38% of unreacted **4b** was recovered.[9b]

The Davoll modification[6] of the Fischer–Helferich nucleoside synthesis[7] (Chart I) was not used for the synthesis of **1b**, but in view of the poor yield obtained by the fusion procedure, the former route should prove the better method of synthesis of this analog.

2-Methoxy-N^6-methyladenosine (**1c**) was prepared from **1a** by metathetical exchange with sodium methoxide in refluxing methanol.

Biological Results.—Preliminary pharmacological evaluation[12] of these compounds as vasodepressor agents in the cat has shown that the compounds are less potent than 2-chloroadenosine or adenosine; they are, however, longer lasting in their effects than is adenosine. In the adenosine diphosphate mediated aggregation of sheep platelets the methylated analogs were found to have only 1–4% of the activity of adenosine but again the duration of the effect was always greater than that of adenosine. The potencies of the analogs relative to that of adenosine are listed in Table I.

TABLE I

RELATIVE POTENCY OF N^6-METHYLATED ADENOSINE ANALOGS

Derivative of adenosine	Inhib[a] of ADP[b]-induced aggregation of sheep platelets	Vasodepressor effect in the cat[c]
Adenosine	1	1
2-Chloro-	40	50
2-Chloro-N^6-methyl-	0.04	2
2-Methylthio-N^6-methyl-	0.015	0.5
2-Ethylthio-N^6-mechyl-	0.005	0.5
2-Methoxy-N^6-methyl-	0.01	0.5
2-Trifluoromethyl-N^6-mechyl-	0.01	0.2
2-Chloro-N^6-dimethyl-	0.02	0.2

[a] Estimated by the turbidimetric method of G. V. R. Born [*Nature*, **194**, 927 (1962)]. Adenosine was used as a reference compound. [b] ADP = adenosine diphosphate. [c] Analogs were dissolved in normal saline and administered in the jugular vein of cats anesthetized with pentobarbital. Blood pressure was recorded from the left common carotid artery with a Statham pressure transducer coupled to a chart recorder. Adenosine was used as a reference compound.

Three of the analogs, **1a**, **1b**, and **1e** have been shown to be potent inhibitors of cardiac adenosine deaminase, more potent than their corresponding nonmethylated analogs, 2-chloroadenosine, 2-methylthioadenosine, and 2-trifluoromethyladenosine. These results are reported in detail elsewhere.[13]

(11) A. Albert and D. J. Brown, *J. Chem. Soc.*, 2060 (1954).

(12) The authors are indebted to Mr. F. Michal of this Department for these results.

Experimental Section[14]

2-Ethylthio-6-hydroxypurine.—To a solution of 48 g (0.28 mole) of 2-thio-6-hydroxypurine[15] in 240 ml of 2 *N* NaOH and 120 ml of water, 62 g (0.4 mole) of diethyl sulfate was added dropwise with stirring. The temperature was maintained at 28–35° and stirring was continued for 90 min. The reaction mixture was allowed to stand at room temperature overnight, then adjusted to pH 5 with 50% acetic acid, and cooled. 2-Ethylthio-6-hydroxypurine separated and was filtered and recrystallized from water to give 32 g of a pale yellow crystalline solid. The reaction was repeated and the combined products were dried for 24 hr *in vacuo* at 130°, to give 64 g of material: $\lambda_{max}^{pH\ 1}$ 264 mμ; $\lambda_{max}^{pH\ 13}$ 271, 227 mμ. The product was recrystallized several times from methanol as a white microcrystalline powder, mp 254–255°, but satisfactory analyses could not be obtained.

2-Ethylthio-6-chloropurine.—2-Ethylthio-6-hydroxypurine (60 g, 0.31 mole), freshly distilled $POCl_3$ (900 ml), and freshly distilled anhydrous diethylaniline (90 ml, 0.565 mole) were heated together under reflux for 45 min. Excess $POCl_3$ was evaporated, and the viscous residue was poured into ice water (400 ml) with stirring. A dark sticky mass formed, the supernatant was decanted, and the tar was repeatedly triturated with water until no further solution of tarry material could be obtained. The combined aqueous solutions were filtered and extracted with diethyl ether for 8 hr in a liquid–liquid extractor. Evaporation of ether left a yellow solid which was recrystallized from ethanol to give 28 g (42%) of a pale yellow crystalline solid. Several recrystallizations from ethanol gave the analytically pure material: white cubes, mp 185–187°; $\lambda_{max}^{pH\ 6}$ 306, 258.5 mμ; $\lambda_{max}^{pH\ 13}$ 304, 243.5 mμ.

Anal. Calcd for $C_7H_7ClN_4S$: C, 39.16; H, 3.29. Found: C, 39.14; H, 3.43.

2-Ethylthio-6-methylaminopurine.—2-Ethylthio-6-chloropurine (10 g) was heated under reflux for 4 hr with 25% aqueous $MeNH_2$ (350 ml). A pale yellow solid separated and was filtered from the cooled solution. The filtrate was evaporated to half volume and cooled. More solid separated and was filtered, yielding a total of 9.9 g. Recrystallization of this from methanol gave 9.1 g of off-white crystals, which sublimed from 180° and melted at 292–294°. An aliquot from a second preparation was recrystallized from water and from methanol to give pure white spindles which sublimed from 180° and melted at 293–294°: $\lambda_{max}^{pH\ 6}$ 287, 250 mμ; $\lambda_{max}^{pH\ 13}$ 283, 233 mμ.

Anal. Calcd for $C_8H_{11}N_5S$: C, 45.91; H, 5.30. Found: C, 46.11; H, 5.35.

2-Trifluoromethyl-6-methylaminopurine.—2-Trifluoromethyl-6-chloropurine[16] (5 g) was heated under reflux with 30% aqueous $MeNH_2$ (200 ml) for 2 hr. A white solid separated and was filtered; the filtrate was concentrated to half volume and yielded a second crop of solid giving a total of 4.65 g which sublimed from 210° and decomposed at 280°. Recrystallization from Methyl Cellosolve gave 4.15 g (85%). Two recrystallizations from methanol of an aliquot from a second preparation gave a pure white microcrystalline solid, which volatilized without melting at 282°; $\lambda_{max}^{pH\ 1}$ 273 mμ; $\lambda_{max}^{pH\ 13}$ 272, 222.5 mμ.

Anal. Calcd for $C_7H_6F_3N_5$: C, 38.72; H, 2.79. Found: C, 38.92; H, 3.19.

2-Chloro-N^6-methyladenosine (1a). (i).—A 10% aqueous solution of NaOH (16 ml) was added to a stirred suspension of 2-chloro-6-methylaminopurine[17] (7.3 g, 40 mmoles) and Celite (14.5 g) in 350 ml of water, followed by slow addition of a solution

(13) M. Rockwell and M. H. Maguire, *J. Med. Pharm.*, in press.

(14) Melting points were determined on a Kofler Reichert apparatus and are uncorrected. Ultraviolet spectra were obtained on a Perkin-Elmer Model 350 spectrophotometer, and optical rotations were measured on a Hilger polarimeter. Spectral data and optical rotations with standard errors are summarized in Table II. Paper chromatography was carried out by the ascending technique in BuOH–H_2O (86:14), and spots were located by observation under ultraviolet light. Microanalyses were done by the Australian Microanalytical Service, Division of Organic Chemistry, C.S.I.R.O., University of Melbourne.

(15) A. G. Beaman, *J. Am. Chem. Soc.*, **76**, 5033 (1954).

(16) H. Nagaro, S. Irvine, A. Saggiomo, and E. Nodiff, *J. Med. Chem.*, **7**, 125 (1964).

(17) J. A. Montgomery and L. B. Holmes, *J. Am. Chem. Soc.*, **80**, 401 (1958).

TABLE II
SPECTRAL DATA AND OPTICAL ROTATIONS

Derivative of adenosine	λ_{max}, mμ (ε × 10⁻³) pH 1	λ_{max}, mμ (ε × 10⁻³) pH 13	$[\alpha]^{21.5}D$, deg (c, g/100 ml, MeOH)
2-Chloro-N^6-methyl-	270(15.5)	268(16.8)	−53.5 ± 0.2(0.3355)
2-Chloro-N^6-dimethyl-	278(18.7)	276(19.6)	−58.6 ± 0.2(0.2937)
2-Methylthio-N^6-methyl-	272(15.8)	279(15.9), 239(19.8)	−40.2 ± 0.2(0.9877)
2-Ethylthio-N^6-methyl-	272.5(17.1)	280(17.2)240.5(21.3)	−34.6 ± 0.2(1.032)
2-Trifluoromethyl-N^6-methyl- (+ 0.5H_2O)	266(13.0)	265(13.4)	−48.8 ± 0.2(0.9688)
2-Methoxy-N^6-methyl- (+ H_2O)	276.5(14.1)	269(16.2)	−46.6 ± 0.2(1.013)

of $HgCl_2$ (10.86 g, 40 mmoles) in ethanol (350 ml). A gel-like suspension of the chloromercuri derivative gradually formed; the pH was adjusted to 8 with aqueous NaOH, and the suspension was kept at room temperature for 12 hr. The product was filtered, washed with water and ethanol, and dried *in vacuo*. The material (29.9 g, 94%) was added to anhydrous xylene (600 ml) and further dried by azeotroping under a Dean–Stark head. A xylene (50 ml) solution of **3**,[18] which was prepared from 20.16 g (40 mmoles) of **5**,[19] was added and the mixture was refluxed and stirred for 3 hr. The hot suspension was filtered and the filtrate was evaporated leaving an oil which was dissolved in $CHCl_3$ (100 ml). The filter cake was washed with three 100-ml portions of hot $CHCl_3$. The chloroform solutions were combined and extracted with two 100-ml portions of 30% aqueous KI and two 100-ml portions of water and dried (Na_2SO_4). Chloroform was evaporated leaving a viscous orange oil, which was kept for 2 days at 2° with absolute methanol (100 ml) saturated with NH_3. The oil gradually dissolved. Methanol was evaporated leaving a brown oil (10.0 g). This was partitioned between water (50 ml) and $CHCl_3$ (50 ml.). The aqueous extract was extracted once more ($CHCl_3$) and evaporated to a clear brownish glass, which crystallized when triturated with ethanol. Filtration yielded 6.1 g of brown crystals, and two recrystallizations from methanol gave 4.3 g (34%) of **1a**. Two more recrystallizations from methanol gave pure **1a**, mp 203–204°. The spectral data are recorded in Table II.

Anal. Calcd for $C_{11}H_{14}ClN_5O_4$: C, 41.84; H, 4.47; N, 22.19. Found: C, 41.88; H, 4.82; N, 21.83.

(ii).—A mixture of 2,6-dichloropurine[20] (18.9 g, 0.1 mole) and **5** (50.4 g, 0.1 mole) was heated *in vacuo* at 135° in a rotating flask until a clear melt was obtained. Considerable evolution of acetic acid occurred at this stage. The reaction flask was cooled to room temperature, anhydrous *p*-toluenesulfonic acid (200 mg) was added, and the flask again was heated *in vacuo* with rotation at 140° for 25 min. A vigorous gas evolution occurred and a clear glass was obtained. The flask was cooled to room temperature and the product was dissolved in $CHCl_3$ (500 ml). The chloroform solution was washed with three 200-ml portions of saturated aqueous $NaHCO_3$ and with two 100-ml portions of water, filtered, and dried (N_2SO_4). Evaporation of $CHCl_3$ left a clear pale yellow glass which was triturated with hexane to give the blocked 2,6-dichloropurineriboside as a hexane-insoluble cream powder (60 g). Thin layer chromatography on silica gel in $CHCl_3$–ethyl acetate (9:1) showed that the blocked nucleoside was essentially homogeneous, and it was not further purified. Five grams of this product was treated with 300 ml of anhydrous methanol, and the mixture was saturated with anhydrous methylamine at 0°. It was kept at 3° for 3 days and the solid gradually dissolved. The solution was then transferred to an autoclave and kept for 2 days at room temperature. Methanol was evaporated leaving a clear brown glass which was dissolved in water (50 ml). The aqueous solution was extracted with three 20-ml portions of $CHCl_3$ and evaporated to a viscous brown oil. Trituration of this with methanol gave brownish crystals (1.34 g) which were recrystallized from ethanol to give 1.1 g of **1a**, mp 205–207°. A mixture melting point with the product obtained by route i and comparison of the spectral properties of the two products showed them to be identical.

2-Methylthio-N^6-methyladenosine (**1b**). (i).—2-Methylthio-6-chloropurine[21] (2.0 g, 9.9 mmoles) was heated *in vacuo* with 5.04 g (10 mmoles) of **5** to 140° without appreciable solution of the purine. Approximately 30 mg of dry *p*-toluenesulfonic acid was added to the cooled melt and the mixture was reheated to 130–140°. A brisk effervescence occurred and after 35 min at 140° a clear melt was obtained. This was cooled to give a glass which was dissolved in $CHCl_3$ (100 ml). The $CHCl_3$ solution was washed with saturated aqueous $NaHCO_3$ (50 ml) then with two 75-ml portions of H_2O and dried (Na_2SO_4). Chloroform was evaporated leaving a brown glass (5.7 g). This was dissolved in 250 ml of anhydrous methanol, and the solution was saturated with dry methylamine at 0° and allowed to stand for 3 days in an autoclave at room temperature. MeOH was evaporated to give a dark residue. This was triturated with acetone (100 ml), and the acetone solution was decanted from some black tarry material and evaporated to a dark oil which was partitioned between $CHCl_3$ and water (100 ml each). The aqueous layer was treated with charcoal, filtered, evaporated to 10 ml, and cooled in ice. Crystals separated and were filtered to yield 0.55 g (17%) of brownish prisms, mp 113–115°, which were purified as described below.

(ii).—2-Methylthio-6-chloropurine (3.5 g, 17.4 mmoles) was heated under reflux for 30 min with acetic anhydride (350 ml) and the mixture was evaporated to dryness. The residue crystallized from a chloroform solution as needles (3.95 g), mp 142–145°. The acetylated purine (3.95 g) was heated *in vacuo* with **5** (8.82 g) at 130–140° as described above and dissolved to give a clear melt. This was cooled, *p*-toluenesulfonic acid (30 mg) was added, and the mixture was again heated *in vacuo* at 130–140° when a brisk effervescence occurred and the melt darkened considerably. The product was cooled and dissolved in $CHCl_3$ (100 ml). The $CHCl_3$ solution was extracted with saturated aqueous $NaHCO_3$ (50 ml) and with two 50-ml portions of water and dried (Na_2SO_4). $CHCl_3$ was evaporated leaving a dark residue. This was triturated with methanol (100 ml) to give a dark solution and a black insoluble solid. The methanol solution was decanted from the solid (0.98 g) and treated with charcoal, filtered, and evaporated to give a pale yellow residue. This was treated with $CHCl_3$ (100 ml) and 1.35 g of a white insoluble solid was filtered off; melting point and spectral data showed this to be recovered 2-methylthio-6-chloropurine. Chloroform was evaporated from the filtrate, and trituration of the residue with three 50-ml portions of hexane gave 8 g of a solid which was dried *in vacuo* over P_2O_5. The dried solid was dissolved in anhydrous methanol (300 ml), and the solution was saturated at 0° with dry $MeNH_2$ and allowed to stand for 7 days in an autoclave at room temperature. Methanol was evaporated leaving a dark brown glass. This was triturated with acetone and the acetone solution was decanted from a black tar and evaporated to a dark residue, which was dissolved in water (100 ml) and extracted ($CHCl_3$, 100 ml). The aqueous layer was evaporated to 25 ml and slow evaporation of this solution in air at room temperature yielded 0.85 g of **1b** (15%) as brown crystals, mp 115–120°.

The brown crystalline products from the two above preparations were combined and recrystallized three times from 50% aqueous methanol to yield 0.9 g of white needles, mp 172–174°. A further recrystallization from water gave 0.80 g of white prisms, mp 118–119° (presumably a hydrate), which were finally crystallized from methanol as 0.67 g white needles, mp 170–171°.

Anal. Calcd for $C_{12}H_{17}N_5O_4S$: C, 44.03; H, 5.23, N, 21.39. Found: C, 44.00; H, 5.26; N, 21.27.

2-Ethylthio-N^6-methyladenosine (**1d**).—2-Ethylthio-6-methylaminopurine (9.1 g, 43.5 mmoles) and Celite (20 g) were ground to a fine powder. The mixture was added to a vigorously stirred solution of $HgCl_2$ (11.8 g, 43.5 mmoles) in 50% ethanol (500 ml). A solution of NaOH (1.75 g, 43.7 mmoles) in water (40 ml) was added dropwise to the stirred suspension which

(18) H. M. Kissman, C. Pidacks, and B. R. Baker, *J. Am. Chem. Soc.*, **77**, 18 (1955).

(19) E. F. Recondo and H. Rinderknecht, *Helv. Chim. Acta*, **42**, 1171 (1959).

(20) A. G. Beaman and R. K. Robins, *J. Appl. Chem.*, **12**, 432 (1962).

(21) C. W. Noell and R. K. Robins, *J. Org. Chem.*, **24**, 320 (1959).

gradually became yellow. Stirring was continued for 30 min, then the Celite–chloromercuri-2-ethylthio-6-methylaminopurine mixture was filtered, washed with ethanol, and dried *in vacuo* (yield 39.28 g, 99%). The product was further dried and treated with 43.5 mmoles of **3** as described for **1a**. The condensation product was deblocked with methanolic NH_3 as described for **1a**, and **1d** (6.2 g) crystallized during the chloroform extraction of the final aqueous solution. Two recrystallizations from ethanol gave white plates (4.4 g, 30%), mp 206–207.5°.

Anal. Calcd for $C_{13}H_{19}N_5O_4S$: C, 45.73; H, 5.61; N, 20.51. Found: C, 45.79; H, 5.81; N, 20.00.

2-Trifluoromethyl-N^6-methyladenosine (1e).—Chloromercuri-2-trifluoromethyl-6-methylaminopurine on Celite was prepared from 4.12 g (19 mmoles) of the purine, 10 g of Celite, 5.18 g (19 mmoles) of $HgCl_2$, and 0.765 g (19 mmoles) of NaOH as described for **2d**; 17.2 g of the chloromercuri-2-trifluoromethyl-6-methylaminopurine–Celite mixture was obtained and treated with 19 mmoles of **3** (from 9.65 g, 19 mmoles, of **5**). The procedure and work-up were as described for **1d**, and **1e** separated from water as a light brown crystalline solid (2.85 g, 43%), mp 203–205°, which crystallized from water as white needles (2.4 g, 36%), mp 203.5–206°. A second recrystallization gave pure **1e**, mp 204–206°.

Anal. Calcd for $C_{12}H_{14}F_3N_5O_4 \cdot 0.5H_2O$: C, 40.19; H, 4.19; N, 19.54. Found: C, 40.15; H, 4.20; N, 19.49.

2-Chloro-N^6-dimethyladenosine (1f).—A chloromercuri-2-chloro-6-dimethylaminopurine–Celite mixture was prepared in 84% yield, as described for **2d** from 6.0 g (30.4 mmoles) of 2-chloro-6-dimethylaminopurine,[17] Celite (12.0 g), $HgCl_2$ (8.25 g, 30.4 mmoles), and NaOH (1.22 g, 30.4 mmoles). The dried product (21.6 g) was treated with 30.4 mmoles of **3** (from 15.3 g, 30.4 mmoles, of **5**) as described for 1d. Evaporation of methanolic NH_3 after the deblocking step left a dark oil. Trituration with $CHCl_3$–water gave crystals (4.55 g, 46%) which were recrystallized twice from aqueous ethanol to give **1f** as needles (3.5 g), mp 214–215°. Two recrystallizations from methanol gave analytically pure **1f**, mp 218–220°.

Anal. Calcd for $C_{12}H_{10}ClN_5O_4$: C, 43.69; H, 4.89; N, 21.24. Found: C, 44.02; H, 5.08; N, 21.27.

2-Methoxy-N^6-methyladenosine (1c).—Sodium methoxide (2 *N*, 8 ml) was added to a solution of **1a** (1.0 g) in dry methanol (100 ml), and the solution was heated under reflux for 50 hr. Paper chromatography showed that only a trace of **1a** remained. The solution was cooled, neutralized with dilute HCl, and evaporated to a white residue. This was extracted with dry ethanol (50 ml), NaCl was filtered off, and the filtrate was evaporated leaving a colorless gum. This was dissolved in water (50 ml), and the solution was filtered from some insoluble material, concentrated to 10 ml, and refrigerated. Crystals of **1c** (0.75 g) separated. The product was chromatographically pure and two recrystallizations from 50% aqueous ethanol gave pure **1c**, clusters of needles, mp 106–108°.

Anal. Calcd for $C_{12}H_{17}N_5O_5 \cdot H_2O$: C, 43.77; H, 5.82; N, 21.27. Found: C, 43.93; H, 6.21; N, 21.22.

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Unnatural Amino Acids. II. Congeners of DL-3-Carboxy-4-methoxyphenylalanine

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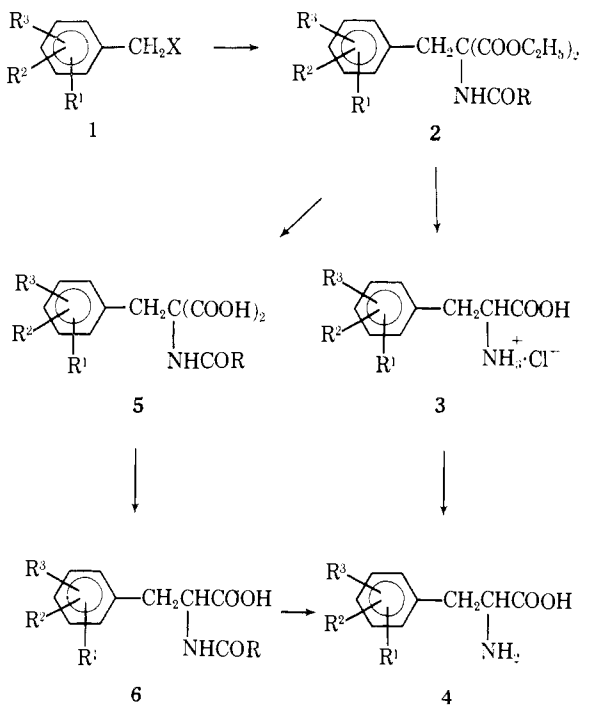
Several studies on the use of amino acid residues to transport biologically active groups across cell membranes have been reported.[2-6] In the main, however, the possibility that an amino acid derivative of a pharmacologically active moiety would penetrate to sites not reached by the parent compound has not been explored. In the first paper of this series,[6] the preparation and some of the biological properties were described of three new amino acids related both to tyrosine and aspirin. One of the compounds, DL-3-carboxy-4-methoxyphenylalanine (CMPA; **4**, R^1 = 3-COOH; R^2 = 4-CH_3O; R^3 = H) was found to be an orally effective analgetic agent in animals and man but was poorly and erratically absorbed. It served, however, as a reference compound in the search for compounds which are better absorbed and at least as potent. The congeners of DL-3-carboxy-4-methoxyphenylalanine which were prepared and evaluated (Tables I and II) include isomers, homologs, and analogs. All were synthesized from acylamidomalonates as shown in Scheme I.

SCHEME I



The pharmacological data in Table I show that small structural changes in DL-3-carboxy-4-methoxyphenylalanine (CMPA) are accompanied by increased toxicity and attenuation or loss of analgetic activity. Replacement of the methoxy group by ethoxy or chloro and of the carboxy group by formyl or carboxamido results in considerable loss of analgetic activity. Substitution of the benzene nucleus of CMPA with a methyl group does not seem to have a markedly deleterious effect on analgetic activity but causes a great increase in toxicity. One of the isomers of CMPA, DL-3-carboxy-2-methoxyphenylalanine, shows analgetic activity in the same range as CMPA but is more toxic.

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