and treated with charcoal. The hydrochloride **14** was prepd and recrystd from EtOH–Et_2O to give 0.49 g (28%), mp 218–220° dec.

4-[3-(2-Chlorophenothiazin-10-yl)propyl]-4-azahomoadamantane·HCl (15) was prepd from **2** analogously to the method of Grogan, *et al.*[21]

4-*p*-Tolylsulfonylcarbamoyl-4-azahomoadamantane (16) was prepd from **2** according to the method described by Gerzon, *et al.*[2a]

5-Hydrazino-4-azahomoadamant-4-ene·HCl (18).—A mixt of **3a** (2.7 g, 0.015 mole) and hydrazine hydrate (1.0 g, 0.02 mole) was stirred at room temp for 48 hr. Then CH_2Cl_2 (25 ml) was added, and the resulting soln was dried and evapd *in vacuo*. The residue was treated with ethanolic HCl and Et_2O to yield **18**, 2.26 g (70%), mp 287–290° dec.

1-(4-Azahomoadamant-5-ylidene)-3-phenylguanidine·2HCl (23).—A mixt of **22** (2.2 g, 11.6 mmoles) and $PhNH_2$·HCl (1.5 g, 11.6 mmoles) in DMSO (25 ml) was heated on a steam bath for 17 hr and then concd to dryness. The residue was dissolved in a mixt of H_2O (50 ml) and 2 *N* HCl (6 ml). The soln was washed with Et_2O (3 × 15 ml). The aq layer was filtered (Hyflo Supercel), made alk, and extd with Et_2O (4 × 30 ml). These exts were combined, dried, and concd. The residue was treated with ethanolic HCl and Et_2O to give **23**, yield 2.32 g (50%), mp 134–142° dec. According to the nmr spectrum and the elemental analysis the compd contained 1 mole of EtOH.

4-Azahomoadamantano[5,4-*b*]quinazolin-4′(3′*H*)-one (39).—The lactam **1** (3.50 g, 21 mmoles) and isatoic anhydride (3.55 g, 22 mmoles) were mixed thoroughly and then heated in a metal bath to approx 160°. After melting, the mixt was kept at this temp for 10 min and then the temp was raised to 190° over a period of 5 min. After the evolution of CO_2 had ceased, the residue was cooled and dissolved in concd HCl (60 ml). This soln was treated with charcoal and filtered (Hyflo Supercel). The filtrate was made alk with 50% NaOH. The first few milliliters caused the sepn of some tarry material, which was discarded. Further addn of NaOH gave a white ppt which was filtered, washed with H_2O, and dried. Recrystn from EtOH–H_2O (1:1) yielded 1.89 g (33%), mp 165–167°.

6′,8′-Dichloro-4-azahomoadamantano[5,4-*b*]quinazolin-4′(3′*H*)-one (40).—A mixt of **1** (3.0 g, 18 mmoles) and 3,5-dichloroisatoic anhydride[22] (4.5 g, 19 mmoles) was heated at 165° for 10 min. Then the temp was raised to 190°, and the mixt was kept at this temp for 45 min. After cooling, the residue was successively extd with 100, 25, and 25 ml of boiling EtOH. On cooling the combined exts yielded a ppt which was recrystd from EtOH; yield 1.17 g (20%), mp 217–220°.

4-Azahomoadamantano[5,4-*b*]-3′,4′-dihydroquinazoline (41).—Compd **39** (3.7 g, 0.014 mole), dissolved in dry Et_2O (275 ml), was added slowly to a soln of LAH (1.4 g, 0.037 mole) in dry Et_2O (75 ml). After being refluxed for 7 hr the mixt was worked up in the usual way; yield, after recrystn from MeOH (50 ml), 2.38 g (60%), mp 148–155°. The product contained 1 mole of MeOH.

4-Azahomoadamantano[5,4-*b*]-1′,2′,3′,4′-tetrahydroquinazoline·HCl (42).—Compd **39** (5.9 g, 0.022 mole) was reduced with LAH (3.0 g, 0.080 mole) in boiling THF (50 ml) (reaction time 64 hr), and the reaction mixt was worked up as usual. The base [yield 5.2 g (92%), mp 132.5–135°] was converted into **42**, mp 202–203.5° dec.

4′-Hydroxy-4-azahomoadamantano[4,5-*a*]pyrimidin-6′(1′*H*)-one (43).—To a soln of Na (0.5 g, 0.022 g-atom) in EtOH (15 ml) were added diethyl malonate (1.8 g, 0.011 mole) and **17**·HCl (2.01 g, 0.010 mole). The mixt was stirred and refluxed for 5 hr. After evapn of the solvent, H_2O (10 ml) was added, and the soln was acidified (pH 4) with 2 *N* HCl. The resulting ppt was filtered off and washed with H_2O. After recrystn from H_2O, 0.9 g (31%) of **43**, contg 3 moles of H_2O, was obtained, mp 264–269°.

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(21) C. H. Grogan, R. Kelly, and L. M. Rice, *J. Med. Chem.*, **9**, 654 (1966).

(22) R. Dorsch, *J. Prakt. Chem.*, **33**, 45 (1886).

2-Alkylthioadenosines, Specific Coronary Vasodilators

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2-Methylthio-, 2-ethylthio-, 2-*n*-propylthio-, and 2-isopropylthioadenosines were synthesized in almost quantitative yield from 2-chloroadenosine by nucleophilic displacement of Cl by the appropriate sodium alkylmercaptide in anhyd DMF. 2-Methylthioadenosine was also synthesized in 45% overall yield from 2-methylthioadenine by the chloromercuri procedure; 2-ethylthioadenosine was obtained similarly from 2-ethylthioadenine, and from 2-ethylthio-6-chloropurine by fusion with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose. 2-*n*-Propylthioadenosine and 2-isopropylthioadenosine were also synthesized by the fusion method from 2-*n*-propylthio-6-chloropurine and 2-isopropylthio-6-chloropurine, respectively. The four 2-alkylthioadenosines had coronary vasodilator activity in the dog of prolonged duration. The coronary vasodilator potency of the compounds increased with increasing length of the alkyl chain; the isopropylthio derivative was slightly less potent than 2-*n*-propylthioadenosine. Doses of the 2-alkylthioadenosines which caused coronary vasodilatation had no depressant effect on heart rate and contractility.

The effects of adenosine on the mammalian cardiovascular system, which were first described by Drury and Szent-Györgi in 1929, include a transient increase in coronary blood flow, a transient decrease in systemic blood pressure, sinus brachycardia, and arterioventricular block.[1] The dose of adenosine which slows the heart rate is greater than the dose which causes a threshold increase in coronary blood flow, but even low doses of adenosine may have a depressant effect on the heart.[2] The use of adenosine in cardiovascular therapy has been precluded both by the transitory nature of its vasodilator effects and by its toxic actions on the heart. It would seem feasible however that certain analogs of adenosine may be found which have the coronary vasodilatory activity of adenosine, but which have greater duration of action *in vivo* and which lack the cardiac depressant action of the parent compound. Clarke,

(1) (a) A. N. Drury and A. Szent-Györgi, *J. Physiol.*, **68**, 213 (1929). (b) M. M. Winbury, D. H. Papierski, M. L. Hemmer, and W. E. Hambourger, *J. Pharmacol. Exp. Ther.*, **109**, 255 (1953). (c) J. N. James, *ibid.*, **149**, 233 (1965).

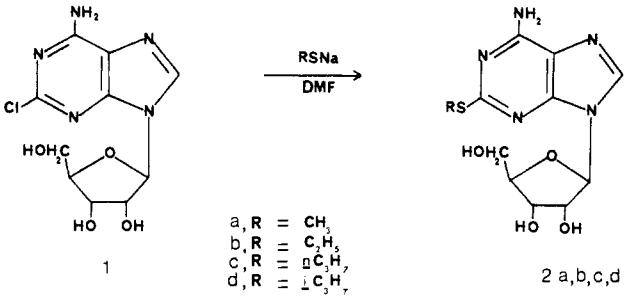
(2) H. Schöndorf, W. Rummel, and K. Pfleger, *Experentia*, **25**, 44 (1969).

et al.,[3] reported the hypotensive effects in the cat of a number of 2-substituted analogs of adenosine, including 2-methylthioadenosine which was considerably less potent as a vasodilator than adenosine, but which in large doses produced prolonged vasodilatation.

Recently further studies of the vasodilatory effects of 2-methylthioadenosine in the anesthetized dog, rat, and guinea pig[4] showed that it had negligible toxic effects on the heart in these species. These findings lead us to synthesize the higher homologs, 2-ethylthio-, 2-*n*-propylthio-, and 2-isopropylthioadenosines and to study their coronary vasodilator activity.

Chemistry.—The four 2-alkylthioadenosines, **2a–2d**, were readily synthesized from 2-chloroadenosine (**1**) by reaction with the appropriate sodium alkylmercaptides (Scheme I). 2-Methylthioadenosine has been

SCHEME I

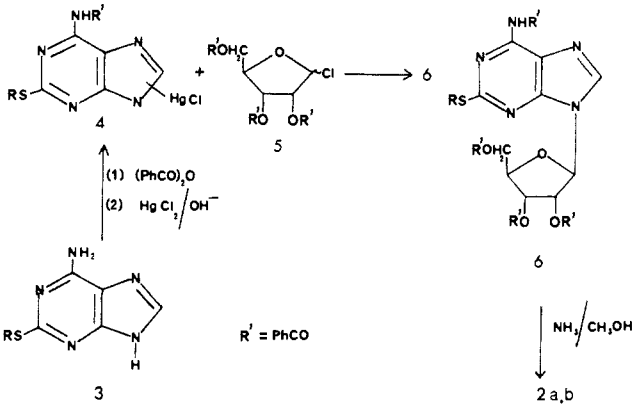


isolated in 38% yield from **1** by reaction with NaSMe in *n*-PrOH,[5] but it was found that when the reaction of **1** with each of the 4 sodium alkylmercaptides was carried out under anhyd conditions in DMF the replacement of Cl^- by alkylmercaptide was complete in 4–7 hr, and the yields of **2a–2d** were almost quantitative. However, as 2-chloroadenosine is a most potent vasodilator,[3,6] it was necessary to ensure by exhaustive paper chromatography that no trace of this analog remained in the 2-alkylthioadenosines, obtained by this route, which were used for pharmacological assay.

When 2-chloroadenosine was not readily available the 2-alkylthioadenosines were synthesized either from the appropriate 2-alkylthioadenines or 2-alkylthio-6-chloropurines. 2-Methylthioadenosine (**2a**) and 2-ethylthioadenosine (**2b**) were obtained from 2-methylthioadenine (**3a**), and 2-ethylthioadenine (**3b**), respectively, by the chloromercuri procedure as outlined in Scheme II. **2a** was first synthesized similarly by Davoll and Lowy[7] *via* 2-methylthio-6-acetamidopurine in 19% overall yield from 2-methylthioadenine.

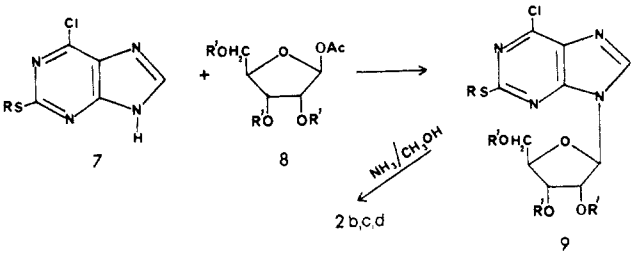
2-Methylthioadenine was synthesized by the amination of 2,6-dimethylthiopurine with 14 *N* NH_4OH at 130–160° for 16 hr, and 2-ethylthioadenine was obtained similarly from 2,6-diethylthiopurine. Both adenines were isolated in good yield, but the yields after purification were often low. A similar difficulty was

SCHEME II



noted by Montgomery, *et al.*,[8] in the purification of 2-methylthio-N^6-dimethylaminopurine obtained from the reaction of 2,6-dimethylthiopurine with aq Me_2NH. An alternative method of preparation of the 2-alkylthioadenines was attempted *via* cyclization of the appropriate alkylisothiouronium isonitrosomalononitrile, a method which Taylor, *et al.*, reported,[9] and which gave a good yield of 2-methylthioadenine. However, while 2-ethylisothiouronium isonitrosomalononitrile was cyclized in refluxing pyridine to give 2-ethylthio-4,6-diamino-5-nitrosopyrimidine, and this on reduction and cyclization with formamide gave **3b**, the overall yield was poor. 2-*n*-Propylthioadenine and 2-isopropylthioadenine, the precursors for the syntheses of **2c** and **2d** by the ClHg procedure, could not be synthesized by this route, as *n*-propylisothiouronium isonitrosomalononitrile could not be crystallized, and the analogous *i*-Pr salt cyclized in very poor yield to give the intermediate 2-isopropylthio-4,6-diamino-5-nitrosopyrimidine. The most facile preparation of 2-*n*-propylthioadenine and 2-isopropylthioadenine would be amination of the appropriate 2-alkylthio-6-chloropurines (**7c** and **7d**), but this was not investigated as **7c** and **7d** were used more directly in the fusion syntheses of **2c** and **2d** (Scheme III).

SCHEME III



Benzoylation of **3a** and **3b** gave the corresponding 2-alkylthio-6-benzamidopurines which were converted into their ClHg derivatives **4a** and **4b**. These were condensed with 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl chloride (**5**) to give the blocked nucleosides **6a** and **6b**. Treatment of **6a** with methanolic NH_3 gave 2-methylthioadenosine (**2a**) in 45% overall yield from **3a**. Tlc showed that **6b** was contaminated with unreacted **3b** and a sugar derivative, which were separated from **6b** by chromatography on alumina. Chromatographically homogeneous **6b** gave 52% of 2-ethylthioadenosine on

(3) D. A. Clarke, J. Davoll, F. S. Philips, and G. B. Brown, *J. Pharmacol. Exp. Ther.*, **106**, 291 (1952).

(4) (a) R. H. Thorp, D. J. Grose, and A. A. Radojevic, unpublished, 1969. (b) F. Michal, M. H. Maguire, and G. Gough, *Nature (London)*, **222**, 1073 (1969).

(5) H. J. Schaeffer and H. J. Thomas, *J. Amer. Chem. Soc.*, **80**, 3738 (1958).

(6) R. H. Thorp and L. B. Cobbin, *Arch. Int. Pharmacodyn. Ther.*, **118**, 95 (1959).

(7) J. Davoll and B. J. Lowy, *J. Amer. Chem. Soc.*, **74**, 1563 (1952).

(8) J. A. Montgomery, L. B. Holum, and T. P. Johnston, *ibid.*, **81**, 3963 (1959).

(9) E. C. Taylor, O. Vogl, and C. C. Cheng, *ibid.*, **81**, 2442 (1959).

debenzoylation with methanolic NH_3, but in contrast to the yield of **2a** obtained in the reaction sequence starting with 2-methylthioadenine, the yield of **2b** from **3b** was only 27%.

Japanese workers have reported the synthesis of **2a** by the acid-catalyzed fusion of 2-methylthio-6-halogenopurine with tetra-*O*-acetyl-β-D-ribofuranose,[10] but in our hands fusion of 2-methylthio-6-chloropurine with tetra-*O*-acetylribofuranose or 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**8**) in the presence of *p*-TsOH was incomplete and accompanied by much decomposition.[11] However, 2-ethylthio-6-chloropurine (**7b**) reacted with **8** on fusion at 160–170° without a catalyst. The product was a yellow gum which was shown by tlc to consist mainly of the blocked nucleoside **9b** together with some unreacted **7b**, **8**, and another unidentified contaminant. Chromatography on an alumina column separated **9b**, which on treatment with methanolic NH_3 at room temp gave 2-ethylthioadenosine in good yield. 2-*n*-Propylthio-6-chloropurine and 2-isopropylthio-6-chloropurine each reacted readily with **8** on fusion at 140° and yielded the blocked nucleosides **9c** and **9d**, which when deblocked and aminated with methanolic NH_3 gave, respectively, 2-*n*-propylthioadenosine (**2c**) and 2-isopropylthioadenosine (**2d**) in good yield.

Pharmacology.—The coronary vasodilator activity of the 2-alkylthioadenosines was studied in open-thorax dogs maintained under Na pentobarbital anesthesia and artificially respired. Coronary blood flow was measured with an electromagnetic flow probe on the left descending coronary artery. Systemic blood pressure was measured from the femoral artery, cardiac contractility was monitored by a Walton Brodie strain gauge sutured to an area supplied by the left descending coronary artery, and the lead II ECG was used to trigger the cardiotachometer for heart-rate recordings. Measurements were recorded simultaneously on a Grass Model 7 polygraph. The analogs dissolved in normal saline were injected either *via* a cannula into the left atrium or into the femoral vein. Dose ranges studied by direct injection into the left atrium were 2-methylthioadenosine, 25–200 μg/kg; 2-ethylthioadenosine and 2-isopropylthioadenosine, 2.5–100 μg/kg; and *n*-propylthioadenosine, 1–50 μg/kg. When the iv mode of administration was used the dose levels were 5–10 times greater.

The coronary vasodilator potencies of the 2-alkylthioadenosines relative to that of adenosine and the duration of the vasodilator effects are summarized in Table I. The increase in chain length of the alkylthio group from C_1 to C_3 was accompanied by an increase in coronary dilator potency, and 2-*n*-propylthioadenosine (**2c**) was 16 times as potent as 2-methylthioadenosine (**2a**). Branching of the C_3 chain, however, in 2-isopropylthioadenosine (**2d**) resulted in 25% less coronary dilator activity than in the straight chain C_3 analog. The coronary dilator effect of equipotent doses of **2a**, **2b**, and **2c** lasted 6 times as long as that of an equipotent dose of adenosine, while the duration of action of the branched chain alkylthio analogs (**2d**) was slightly shorter.

TABLE I

EFFECTS OF 2-ALKYLTHIOADENOSINES ON CORONARY BLOOD FLOW IN THE OPEN-THORAX DOG

Compd	Coronary dilator potency[a]	Duration of action of equipotent doses, min
Adenosine	1	0.5–1
2-Methylthioadenosine (2a)	0.03	6–8
2-Ethylthioadenosine (2b)	0.12 ± 0.02	5–7
2-*n*-Propylthioadenosine (2c)	0.48 ± 0.14	5–7
2-Isopropylthioadenosine (2d)	0.37 ± 0.06	3–5

[a] Mean results (± SE) obtained from the log dose-response curves by comparing the coronary vasodilator effectiveness of the compound administered intraatrially with that of adenosine. Five animals were used for each compound except for 2-methylthioadenosine where 2 animals were used.

At the dose levels of 2-alkylthioadenosines used for intraatrial administration, *i.e.*, up to 100 μg/kg, there was no significant effect on systemic blood pressure or heart rate and no depression of cardiac contractility (*cf.* Figure 1). On iv administration higher doses of **2b**, **2c**, and **2d** of up to 500 μg/kg caused a very prolonged increase in coronary blood flow lasting up to 90 min. At the higher dose levels there were slight falls in systolic pressure and more marked falls in diastolic pressure indicating some peripheral vasodilator effects However, the blood pressure returned to normal well before the coronary blood flow.

Adenosine and a number of 2-substituted analogs of adenosine are inhibitors of ADP-mediated platelet aggregation.[12] 2-Methylthioadenosine has 10% of the activity of adenosine in inhibiting the *in vitro* ADP-mediated aggregation of sheep platelets,[4b] while 2-ethylthio-, 2-*n*-propylthio-, and 2-isopropylthioadenosines have less than 5% of the activity of adenosine in this system.[13] These findings suggest that *in vivo* the latter three analogs, **2b**, **2c**, and **2d**, would not cause any interference in normal blood clotting mechanisms.

Preliminary toxicity studies indicate that the 2-alkylthioadenosines, **2b**, **2c**, and **2d**, are relatively nontoxic. Ip administration to mice of 2-ethylthio-, 2-*n*-propylthio-, and 2-isopropylthioadenosines in doses up to 250 mg/kg caused no observable toxic manifestation.

Discussion

The 2-alkylthioadenosines (**2a–2d**) differed from adenosine not only in the potency and duration of their coronary vasodilator effects in the dog, but also in their lack of toxic cardiac action at higher dose levels. The mechanism by which adenosine causes depression of heart rate and of contractility in many mammalian species is not known, although recent work suggests that the effect on contractility may be due to a direct action of adenosine on the cell membrane, blocking the normal actions of Ca^{2+} in excitation–contraction coupling.[14] Substitution of the alkylthio group in the 2 position of adenosine has removed this component of the cardiovascular action of adenosine, indicating that the 2-alkylthio substituted analogs cannot act at this adenosine site on the cell membrane, unlike, *e.g.*, the 2-

(10) Y. Ishido, Y. Kikuchi, and T. Sato, *Nippon Kagaku Zasshi*, **86**, 240 (1965); *Chem. Abstr.*, **63**, 14963 (1965).

(11) G. Gough and M. H. Maguire, *J. Med. Chem.*, **10**, 475 (1967).

(12) G. V. R. Born, R. J. Haslam, M. Goldman, and R. D. Lowe, *Nature (London)*, **205**, 678 (1965).

(13) F. Michal and F. Penglis, unpublished, 1969.

(14) T. de Gubareff and W. Sleator, *J. Pharmacol. Exp. Ther.*, **148**, 202 (1965).

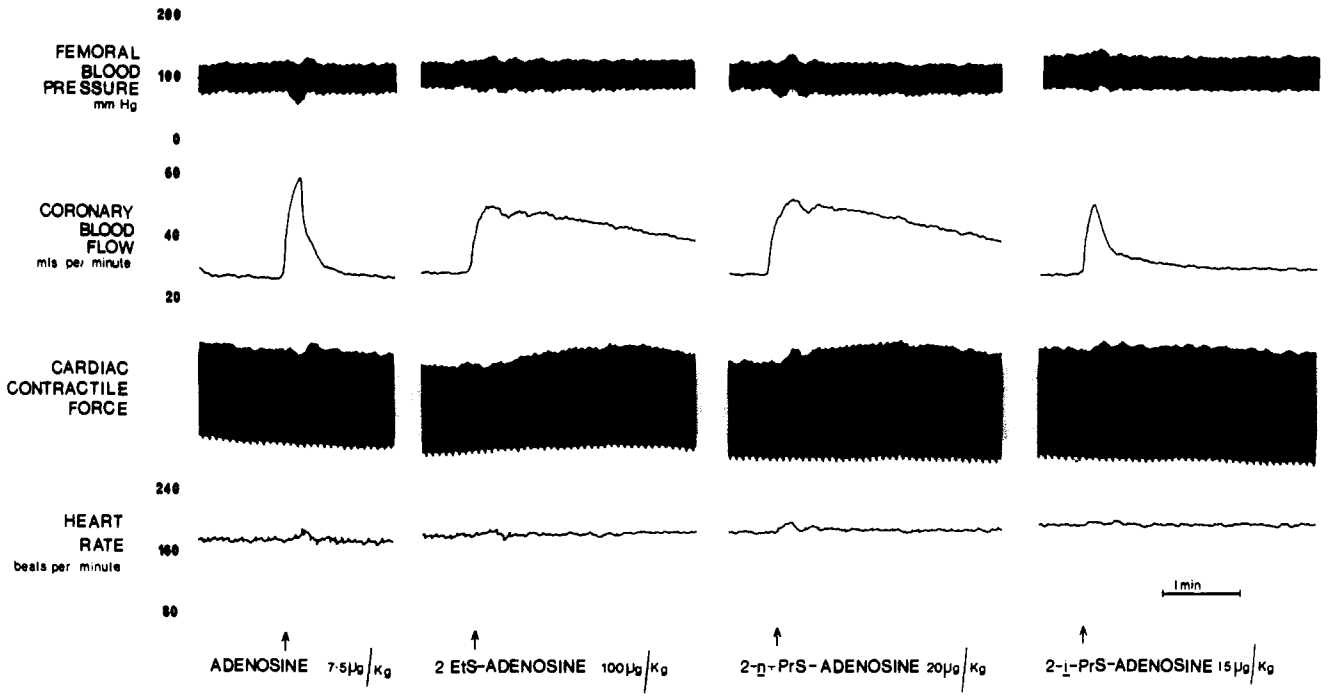


Figure 1.—Effects of intraatrial injections of adenosine, 2-methylthio-, 2-ethylthio-, 2-*n*-propylthio-, and 2-isopropylthioadenosines on femoral arterial blood pressure, coronary blood flow, cardiac contractile force, and heart rate in the open-thorax dog.

TABLE II
ULTRAVIOLET SPECTRAL DATA

Compd	λ_{max}, mμ (ε × 10^{-3}) pH 1	pH 7	pH 13
2,6-Diethylthiopurine	314 (9.8), 261.5 (17.0)	309 (9.9), 259 (17.1)	309 (9.9), 245 (19.9)
2-Ethylthioadenine	282 (10.5), 246.5 (11.3)		280 (9.6), 233.8 (17.5)
2-Ethylthio-6-benzamidopurine	316 (6.6), 260 (32.4)	253 (24.4)	236.5 (22.8)
2-*n*-Propylthio-6-hydroxypurine	264 (14.0)	261 (9.8)	273 (11.3), 228.5 (17.6)
2-Isopropylthio-6-hydroxypurine	265 (15.0)	262 (12.2)	274.5 (11.2), 230 (15.5)
2-*n*-Propylthio-6-chloropurine	305 (5.9), 258.5 (12.7), 231 (15.6)	305 (5.9), 258 (8.9), 232 (15.7)	302.5 (6.0), 242 (19.7)
2-Isopropylthio-6-chloropurine	305 (6.8), 259 (13.0), 231.5 (16.3)	304 (6.1), 259 (8.9), 232 (15.9)	302 (5.9), 243 (17.8)
2-Ethylthioadenosine	268.5 (16.8), 224 (9.8)	275 (14.1), 234 (19.4)	275 (13.8), 234.3 (19.5)
2-*n*-Propylthioadenosine	269 (17.8), 223.5 (10.2)	274.5 (13.4), 235 (18.6)	276 (13.9), 235 (19.5)
2-Isopropylthioadenosine	269 (15.1), 222.5 (8.7)	276 (14.0), 235 (18.8)	275 (14.0), 235 (18.9)

Cl substituted analog which is a more potent myocardial depressant than adenosine.[6]

The transitory nature of the vasodilatory effect of adenosine is believed to be due in part to inactivation of adenosine by deamination to inosine by plasma adenosine deaminase,[15] and also to uptake of adenosine by the myocardium, lungs, and red blood cells.[16] The four 2-alkylthioadenosines **2a–2d**, are not deaminated by adenosine deaminase and are potent inhibitors of the enzyme purified from bovine heart and placenta.[17] The great duration of the coronary dilator effects of the 2-alkylthioadenosines suggests that, unlike adenosine, they are not readily removed from vascular beds by tissue uptake.

The improvement in coronary flow resulting from the administration of small doses of 2-ethylthio-, 2-*n*-propylthio-, and 2-isopropylthioadenosines together with their lack of significant effect on systemic blood pressure, and their lack of toxicity in mice, indicates that these compounds may prove to be useful therapeutic agents for the treatment of conditions characterized by reduced coronary flow.

(15) F. W. Koss, G. Beisenherz, and R. Maerkisch, *Arzneim.-Forsch.*, **11**, 1130 (1962).

(16) K. Pfleger, E. Seifen, and H. Schöndorf, *Biochem. Pharmacol.*, **18**, 43 (1969).

(17) (a) M. Rockwell and M. H. Maguire, *Mol. Pharmacol.*, **2**, 574 (1966). (b) M. K. Sim and M. H. Maguire, unpublished, 1970.

Experimental Section

Melting points were determined on a Kofler–Reichert apparatus and are uncorrected. Uv spectra were run on a Perkin–Elmer Model 350 spectrophotometer, and spectral data are given in Table II. Optical rotations were measured on a Bellingham and Stanley polarimeter. Paper chromatography was carried out by the ascending technique on Whatman No. 1 paper in the following solvent systems: (A) *n*-BuOH–H_2O (86:14), (B) *n*-BuOH–HOAc–H_2O (60:15:25), (C) *i*-Pr_2O–EtOH (25:10) satd with H_2O, (D) *n*-PrOH–1% NH_4OH (2:1), (E) *i*-PrOH–HCl–H_2O (65:18.4:16.6). The spots were located by observation under uv light. R_f values of **2a–d** are listed in Table III. Silica gel G (E. Merck AE Darmstadt) was used for tlc in $CHCl_3$–EtOAc (9:1). Compds were detected by observation under uv light or exposure to I_2 vapor. DMF was distd and stored over CaH_2. Mercaptans were obtained from Fluka or British Drug Houses.

Microanalyses were carried out by Dr. E. Challen, University of New South Wales, and by The Australian Microanalytical Service, Division of Applied Chemistry C.S.I.R.O., Melbourne, Victoria; where analyses are indicated only by symbols of the elements,

TABLE III

PAPER CHROMATOGRAPHY AND R_f VALUES

Compd	R_f Solvent systems A	B	C
2-Methylthioadenosine (2a)	0.33	0.62	0.30
2-Ethylthioadenosine (2b)	0.63	0.73	0.61
2-*n*-Propylthioadenosine (2c)	0.75	0.78	0.80
2-Isopropylthioadenosine (2d)	0.76	0.81	0.80
2-Chloroadenosine (1)	0.45	0.52	0.40

analytical results obtained for those elements were within ±0.4% of the theoretical value.

2,6-Diethylthiopurine.—EtI (9.5 ml, 108 mmoles) was added dropwise with stirring to a suspension of anhyd K_2CO_3 (14.8 g) and 2,6-dithiopurine[18] (10.0 g, 54 mmoles) in DMF (80 ml). A clear soln was obtained. This was poured into H_2O (300 ml) with stirring, and the resulting yellow suspension was acidified to pH 5 with HOAc. The ppt was filtered and dried *in vacuo* giving 12.6 g (97%) of 2,6-diethylthiopurine, R_f 0.97 in E. A sample was recrystd from H_2O for anal., mp 193–5°. *Anal.* ($C_9H_{12}N_4S_2$) C, H, N.

2-Methylthioadenine (3a).—2,6-Dimethylthiopurine[8] (22 g, 104 mmoles) was heated in a stainless steel bomb for 24 hr at 150–160° with 350 ml of NH_4OH satd at 0°. The soln was evapd *in vacuo* to half vol and refrigerated overnight. A grey solid sepd and was filtered and recrystd 3 times from MeOH to give **3a** as colorless plates (10.2 g, 55%), mp 295–297°. Taylor, *et al.*,[9] give mp 295–296° dec. *Anal.* ($C_6H_7N_5S$) C, H, N. In other similar prepns yields of **3a** varied between 35 and 45%.

2-Ethylthioadenine (3b).—Using the procedure described for the prepn of **3a**, 10 g (41.7 mmoles) of 2,6-diethylthiopurine gave 3.7 g (53%) of **3b** as a cream cryst solid, R_f 0.66 in E. A sample was recrystd for anal. from MeOH–H_2O as colorless plates, mp 267–9°. *Anal.* ($C_7H_9N_5S \cdot 0.25H_2O$) C, H, N. In similar prepns yields varied from 25 to 45%.

2-Methylthioadenosine (2a).—2-Methylthio-6-benzamidopurine[10] was prepd in 68% yield from **3a**, and 10.0 g (354 mmoles) was added to 700 ml of 50% aq EtOH contg $HgCl_2$ (9.53 g, 354 mmoles). A 10% aq soln (14.1 ml) of NaOH was added dropwise to the thick stirred suspension to give the pale yellow ClHg deriv **4a**. The mixt was stirred for 2 hr, kept at room temp for 48 hr, and filtered. The product was washed with 50% aq EtOH and dried *in vacuo* over P_2O_5, yielding 17.8 g (98%) of **4a** which was homogeneous on tlc. This material (9 g, 17.3 mmoles) was added to anhyd xylene (450 ml) and dried further by azeotroping under a Dean–Stark head. A xylene soln (150 ml) containing **5**[19] prepd from 10 g (20 mmoles) of **8**[20] was added over 3.5 hr, and the mixt was refluxed and stirred for a further 1.5 hr. The suspension was filtered hot, and the filtrate was kept overnight at room temp and refiltered to remove some fine solid which sepd. Xylene was evapd leaving a viscous brown oil which was dissolved in $CHCl_3$ (250 ml). The $CHCl_3$ soln was washed with three 100-ml portions of 30% KI and with two 100-ml portions of H_2O and dried (Na_2SO_4). $CHCl_3$ was evapd leaving the blocked nucleoside **6a** as a light brown oil which was homogeneous on tlc. This was kept for 2 days at 2° with MeOH (400 ml) satd with NH_3 at 0°. MeOH was evapd *in vacuo* to a brown oil. Extn with three 50-ml portions of hot $CHCl_3$ removed all colored material and left **2a** as a white solid. Recrystn from H_2O gave 3.5 g of pure **2a** (67% from **6a**), mp 157–160°, resolidified from 194° and remelted 228°. Mp after drying *in vacuo* at 100°, 226–227°. Reported mp are 227°,[7] 222–223°,[10] and 153°, resolidified and remelted at 220°.[5] Uv spectral data were identical with reported values.[5]

2-Ethylthio-6-benzamidopurine.—Powd **3b** (5.0 g, 25.6 mmoles) and Bz_2O (17.5 g, 77.4 mmoles) were heated at 160–170° for 30 min. The reaction mixt was cooled, dissolved in EtOH (150 ml), and heated under reflux for 15 min. EtOH was evapd, and the residue was dissolved in EtOH and reevapd to an oil. This was dissolved in EtOAc (20 ml) and **3b** was pptd by addn of Et_2O (150 ml) and filtered (4.75 g). The filtrate was evapd and the residue crystd from PhH–*n*-hexane to yield 3.4 g. The combined products were recrystd from EtOH–H_2O to give 5.7 g (74%) of **3b**: mp 179–180°; R_f 0.82 in E. *Anal.* ($C_{14}H_{13}N_5OS$) C, H, N.

2-Ethylthioadenosine (2b).—(i) The ClHg deriv **4b**, prepd from 5 g (16.7 mmoles) of 2-ethylthio-6-benzamidopurine as described for the prepn of **4a**, was obtained as a cream powder (8.55 g, 96%), homogeneous on tlc. **4b** (1.6 g, 3.0 mmoles) in refluxing xylene (50 ml) was condensed with **5** prepd from 1.51 g (3.0 mmoles) of **8**, and the reaction mixt was worked up as described for **6a** to give the blocked nucleoside **6b** as a light yellow glass (2.08 g). Tlc showed that this product was contaminated with a purine deriv, material which had a slightly lower R_f than **6b**, and a ribose deriv which moved to the solvent front. Crude **6b** (1.88 g) was applied to a column (4.5 × 46 cm) packed with neutral alumina (BDH) in PhH and eluted successively with 200-ml portions of PhH contg 2, 5, 10, 25, and 50% EtOAc, and finally with EtOAc; 15-ml fractions were collected. Fractions 81–125 contained **6b** and were combined and evapd leaving 1.1 g of **6b**, a pale yellow glass which was homogeneous on tlc.

This material (985 mg) was kept for 2.5 days at room temp with 30 ml of MeOH satd with NH_3 at 0°. MeOH was evapd to a yellow oil contg colorless crystals. H_2O (50 ml) was added, and the soln obtained was extd with two 50-ml portions of $CHCl_3$ and evapd to 10 ml. Compd **2b** crystallized as white needles (160 mg). A further 65 mg was recovered from the filtrate, giving a yield of 52% from **6b**, and 27% overall yield from **3b**. The isolation of some **2b** which remained in the aq mother liquor was prevented by the presence of benzamide. The anal. sample of **2b**, obtained by two recrystns from H_2O, was dried *in vacuo* at 100°: mp 211–212°; $[\alpha]_{21.5D}$ − 26.0° (*c* 1.3, DMSO). *Anal.* ($C_{12}H_{17}N_5O_4S \cdot 0.25H_2O$) C, H, N.

(ii) **7b** (1.07 g, 5 mmoles) was fused with **8** (2.52 g, 5 mmoles) *in vacuo* at 160–170° for 35 min. A slow effervescence occurred, and a greenish yellow clear melt was obtained. The cooled product was dissolved in $CHCl_3$ (25 ml), and the $CHCl_3$ soln was washed with three 25-ml portions of satd aq $NaHCO_3$ and two 25-ml portions of H_2O, and dried (Na_2SO_4). Evapn of $CHCl_3$ left a greenish yellow glass (3.42 g) which was shown by tlc to contain the blocked nucleoside **9b**, as the major component, R_f 0.56, together with unreacted sugar which ran to the solvent front, and 4 minor components of low R_f. **9b** (2.38 g) was purified by chromatog on a basic alumina (Woelm) column (4.5 × 45 cm) packed in PhH. Elution was carried out with PhH (400 ml), 200-ml portions of PhH contg 5, 10, 25, and 50% EtOAc, and finally with EtOAc; 15-ml fractions were collected. The ribose contaminant was eluted in fractions 28–75, and **9b** in fractions 101–120, which were combined and evapd giving 1.83 g (75%) of **9b** as a colorless glass, homogeneous on tlc.

This product (200 mg) was kept for 9 days at room temp in MeOH (5 ml) satd with NH_3 at 0°. MeOH was evapd to give a colorless oil which on trituration with hot $CHCl_3$ gave 92 mg of a white solid. This was recrystd from H_2O giving 78 mg (77%) of **2b**, mp 211–212°, homogeneous on chromatog in solvents A, B, and C. The overall yield of **2b** from **7b** was 54%.

2-*n*-Propylthio-6-hydroxypurine.—*n*-PrI (3.7 g, 21.8 mmoles) was added dropwise to a suspension of 2-thio-6-hydroxypurine[15] (3.4 g, 20.2 mmoles) and K_2CO_3 (2.8 g) in DMF (15 ml). The mixt was warmed on a steam bath to initiate reaction, stirred for 20 min at room temp, and then poured into 25 ml of H_2O and ice. The pH was adjusted to 4–5 with HOAc. The product pptd and was filtered, washed with H_2O, and recrystd from EtOH to give 2.3 g (54%) of pale yellow crystals, R_f 0.70 in D. A sample was recrystd for anal. as colorless needles, mp 289°, softening from 285°. *Anal.* ($C_8H_{10}N_4OS$) C, H, N.

2-Isopropylthio-6-hydroxypurine.—*i*-PrI (22 g, 129 mmoles) was added gradually to a suspension of 2-thio-6-hydroxypurine (20 g, 119 mmoles) and K_2CO_3 (15.2 g) in DMF (90 ml). The mixt was heated for 2 hr on a steam bath and the resulting clear soln was worked up as described for the prepn of 2-*n*-propylthio deriv giving 20 g (80%) of 2-isopropylthio-6-hydroxypurine, R_f 0.74 in D. An aliquot was recrystd for anal. from EtOH–H_2O as colorless plates, mp 250°. *Anal.* ($C_8H_{10}N_4OS$) C, H, N.

2-*n*-Propylthio-6-chloropurine (7c).—Freshly distd $POCl_3$ (15 ml) was added to a suspension of 2-*n*-propylthio-6-hydroxypurine (1 g, 4.75 mmoles) in $PhNEt_2$ (1.5 ml), and the mixt was refluxed for 1.25 hr. Excess $POCl_3$ was distd *in vacuo* leaving a light brown oil which was triturated with Et_2O and yielded a yellow solid. This was filtered, washed with ice-cold H_2O until the washings were neutral, and recrystd from EtOH–H_2O giving **7c** as colorless needles (0.75 g, 69%): R_f 0.92 in D; mp 183°. *Anal.* ($C_8H_9ClN_4S \cdot 0.1H_2O$) C, H, N.

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2-Isopropylthio-6-chloropurine (7d).—This was obtained as colorless plates, R_f 0.92 in D, mp 187°, in 59% yield from 2-isopropyl-6-hydroxypurine as described for the prepn of **7c**. *Anal.* ($C_8H_9ClN_4S$) C, H, N.

2-*n*-Propylthioadenosine (2c).—A finely powd mixt of **7c** (104 mg, 0.455 mmole) and **8** (230 mg, 0.455 mmole) was heated *in vacuo* at 140–160° for 20 min. The resulting colorless viscous melt (291 mg) was kept for 10 days in anhyd MeOH (15 ml) satd with NH_3 at 0°. MeOH was evapd, and the cream semisolid residue was extd with 5 ml of boiling hexane–$CHCl_3$ (1:1) and crystd from H_2O (20 ml) to give colorless crystals, which were recrystd from aq EtOH to give **2c** as hydrated plates (129 mg, 80%) of indefinite mp. The anal. sample was obtained by recrystn from H_2O, and dried *in vacuo* at 100° for 8 hr: mp 168°; $[\alpha]_{21.5D}$ − 26.2° (*c* 2.06, DMSO). *Anal.* ($C_{13}H_{19}N_5O_4S \cdot 0.5H_2O$) C, H, N.

2-Isopropylthioadenosine (2d).—A powd mixt of **7d** (2.26 g, 10 mmoles) and **8** (5.04 g, 10 mmoles) was fused *in vacuo* at 140–150° for 35 min, and the product was worked up as described for the reaction of **7b** with **8** to give the blocked nucleoside **9d** as a pale yellow foam (6.95 g). This (1.0 g, 1.5 mmoles) was kept for 9 days at room temp in anhyd CH_3OH (30 ml) satd with NH_3. MeOH was evapd, and the residue was extd with boiling $CHCl_3$ (50 ml) and crystd from H_2O yielding 34.0 mg (66%) of **2d**. Recrystn from EtOH gave the anal. sample: mp 188–189°; $[\alpha]_{21.5D}$ 24.6° (*c* 0.50, DMSO). ($C_{13}H_{19}N_5O_4S$) C, H, N.

2-Alkylthioadenosines from 2-Chloroadenosine.—Finely divided Na (0.35 g, 15 mg-atoms) was added gradually with stirring to 20 ml of Et, *n*-Pr, or *i*-Pr mercaptan at room temp. When reaction was complete (30–60 min), DMF (20 ml) was added, and the mixt was heated at 80–90° to give a clear soln. 1[21] (302 mg, 1 mmole) was added, and the soln was heated at 80–90° for 4–7 hr during which time NaCl sepd. The reaction mixt was cooled, neutralized with HCl, evapd to dryness *in vacuo*, and dried *in vacuo* over P_2O_5. The residue was extd with three 100-ml portions of abs EtOH or (for 2-*n*-propylthioadenosine) *i*-PrOH, and the alcoholic extracts were filtered and evapd to give in each case a colorless glass which crystd from H_2O to give almost quant yields of 2-ethylthio-, 2-*n*-propylthio-, and 2-isopropylthioadenosines (**2b–d**). Paper chromatog of the products in solvents A, B, C, and D with markers of 1 and the appropriate 2-alkylthioadenosine showed no contamination with 1. The prepns were scaled up tenfold without difficulty.

For the synthesis of **2a** from 1, NaSMe was prepd by the gradual addn of Na (1.5 g, 67 mg-atoms) to MeSH (20 g) cooled in a solid CO_2–MeOH bath. The reaction mixt was then kept at room temp while MeSH refluxed from a condenser through which passed MeOH cooled with solid CO_2. The coating of NaSMe which formed on the particles of Na was dissolved by the addition of several 1- to 2-ml portions of DMF. After 1.5 hr Na had completely reacted. DMF (80 ml) was added, and the soln was heated at 80° to evap excess MeSH. 1 (2.0 g, 6.6 mmoles) was added, and the prepn was continued as described in the general procedure yielding 2.0 g of pure cryst **2a** which was shown by paper chromatog in solvents A, B, and C to be uncontaminated by 1.

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Chemistry of Cephalosporin Antibiotics. 23. 2-Methyl- and 2-Methylenecephalosporins

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Cephalosporin sulfoxide esters **4** react with CH_2O under Mannich conditions to give 2-methylene derivatives **6**. Reduction of the exocyclic double bond yields a mixture of isomeric 2-Me compounds (**7**, **8**, and **9**). The double bond, when treated with Br_2, gives dibromide **16a**; with disiamylborane, gives exclusively the 2α-methyl derivative **8** and the corresponding sulfide 11; and with SH compounds, gives 1:1 adducts (**15**). Reduction of the sulfoxides to the sulfides and removal of the ester-protecting group R^3 give 2-substituted cephalosporanic acids which have antibiotic activity.

We have recently investigated the preparation and properties of cephalosporin sulfoxide esters **4** (Scheme I).[1] As part of that program, we were interested in new structural modifications of the cephem molecule and sought to use the doubly activated CH_2 group at C-2 in condensation reactions with CO compounds. Both 3-methyl- and 3-acetoxymethylcephem derivatives were investigated.

Upon treatment of sulfoxide ester **4a** (prepared from **1a** *via* **2a** as outlined in Scheme I[1]) with aq CH_2O and a variety of primary and secondary amine salts under Mannich conditions, a single new crystalline product, 2-methylene sulfoxide **6a**, formed in high yield. The sulfoxide **4a** and *N*,*N*-dimethylformaldimmonium trifluoroacetate[2] under anhyd conditions gave the same 2-methylene sulfoxide **6a**. Evidently the primary Mannich reaction product **5a** is unstable under the reaction conditions and loses the amino group. The amine salt functions only as a catalyst in the condensation. Acidic and basic catalysts, other than primary and secondary amines and their salts, are ineffective. From this evidence we concluded that the condensation is truly a Mannich type. Similarly, the sulfoxide **4c** gave the 2-methylene sulfoxide **6c**.

Surprisingly, the nature of the ester-protecting group R^3 affected the ease of the reaction. Relatively mild conditions (refluxing in *tert*-BuOH–CH_2Cl_2) were used for the cephalosporin sulfoxides **4a** and **4c** which were protected with the electron-withdrawing trichloroethyl group, but more severe conditions (refluxing in DMF–dioxane) were necessary when the electron-donating *tert*-Bu esters **4b** and **4d** were used. The ease of deuterium exchange at the 2 position of various sulfoxide esters (**4**) paralleled the ease of the Mannich reaction.

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