(1639) S. A. Kaplan, R. E. Weinfeld, C. W. Abruzzo, and M. Lewis, *J. Pharm. Sci.*, **61**, 773(1972).
(1640) K. F. Funk, *Acta Biol. Med. Ger.*, **27**, 611(1971); through *Chem. Abstr.*, **76**, 148936z(1972).
(1641) D. D. Reedler, B. M. Jackson, E. N. Brandt, Jr., and J. C. Thompson, *Amer. J. Physiol.*, **222**, 1571(1972).
(1642) H. Roepke, *Advan. Biosci.*, **1**, 61(1967); through *Chem. Abstr.*, **76**, 121434x(1972).
(1643) D. S. Zaharko, *Cancer Chemother. Rep., Part 3*, **3**, 21 (1972); through *Chem. Abstr.*, **77**, 83381p(1972).
(1644) W. C. Werkheiser, *Ann. N.Y. Acad. Sci.*, **186**, 343(1971); through *Chem. Abstr.*, **76**, 121934k(1972).
(1645) D. S. Hewick, *J. Pharm. Pharmacol.*, **24**, 661(1972).
(1646) A. Breckenridge and M. L. Orme, *Life Sci.*, **11**, 337(1972); through *Chem. Abstr.*, **77**, 43227m(1972).
(1647) J. J. Coffey, *J. Pharm. Sci.*, **61**, 138(1972).
(1648) H. B. Hucker, S. C. Stauffer, and S. E. White, *ibid.*, **61**, 1490(1972).
(1649) B. M. Boulos, W. L. Jenkins, and L. E. Davis, *Amer. J. Vet. Res.*, **1972**, 943; through *Chem. Abstr.*, **77**, 56366f(1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received from the * *Pharmacy Research Unit, Pharmaceutical Research and Development Division, The Upjohn Co., Kalamazoo, MI 49001*, and the † *Operations Division, Syntex Laboratories, Inc., Palo Alto, CA 94304*

▲ To whom inquiries should be directed.

RESEARCH ARTICLES

Carbocyclic Analogs of 6-Substituted Purine Ribonucleosides and of Adenosine Ribonucleotides

Y. FULMER SHEALY▲ and JOE D. CLAYTON

Abstract □ New analogs of 6-substituted purine ribonucleosides in which the furanose ring is replaced by a cyclopentane ring were synthesized. The cyclopentane (carbocyclic) analog of 6-chloropurine ribonucleoside was obtained in pure form and was used to prepare analogs having a methylamino, dimethylamino, hydroxylamino, or methoxy group at position 6 of the purine ring. The availability of the pure 6-chloropurine derivative also permitted improved syntheses of the adenosine and 6-(methylthio)purine ribonucleoside analogs, which had been prepared previously. The ribonucleoside analogs having the chloro, methylamino, hydroxylamino, or methoxy group at position 6 were cytotoxic to neoplastic cells (human epidermoid carcinoma No. 2) in culture, but these compounds were less active than the adenosine analog. In tests against leukemia L-1210 in mice, all of the new and the previously synthesized 6-substituted purine ribonucleoside analogs were administered in a single dose (Day 1 or 2), and most were also administered daily (*q.d.* 1–9) at several dose levels. There was no evidence of activity in these tests. The racemic carbocyclic analogs of adenylic acid and of 3′,5′-adenosine monophosphate (cyclic) were prepared from the adenosine analog. These nucleotide analogs were also cytotoxic.

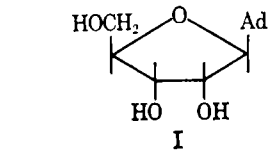
Keyphrases □ Cyclopentane analogs of 6-substituted purine ribonucleosides and adenosine ribonucleotides—synthesis, cytotoxicity □ Carbocyclic analogs of 6-substituted purine nucleosides and adenosine ribonucleotides—synthesis, cytotoxicity □ Purine ribonucleosides, 6-substituted—synthesis of cyclopentane analogs, cytotoxicity □ Adenosine ribonucleotides, 6-substituted—synthesis of cyclopentane analogs, cytotoxicity □ Cytotoxicity—synthesis, evaluation of cyclopentane analogs of 6-substituted purine ribonucleosides and adenosine ribonucleotides

Nucleoside analogs in which a cyclopentane ring replaces the furanose ring may be termed carbocyclic analogs. The racemic[1], carbocyclic analog (II, C-Ado[2]) of adenosine (I) has been synthesized (1) by a multistep route from norbornadiene. The carbocyclic analogs (III–V) of inosine and of the anticancer agents 6-mercaptopurine ribonucleoside and 6-(methylthio)purine ribonucleoside were also prepared (2) from the precursors of II. The carbocyclic analogs of 2′- and 3′-deoxyadenosine were synthesized by similar routes (3); the analog of a pyrimidine deoxyribonucleoside, thymidine, had been prepared earlier by a different method (4).

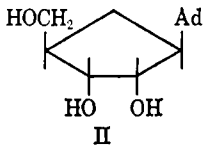
Carbocyclic analogs, having a stable carbon–nitrogen bond instead of a glycosidic bond at position 9 of the purine ring, should not be subject to cleavage by purine nucleoside phosphorylases or hydrolases, but the similarity of their structures to nucleosides endows the carbocyclic analogs with the potential to function either as substrates for, or as inhibitors of, other enzymes that metabolize nucleosides. Biochemical studies show that this potential can, in fact, be realized. Bennett and co-

[1] Structures II–XVI depict one enantiomer of the racemic form that was actually obtained.

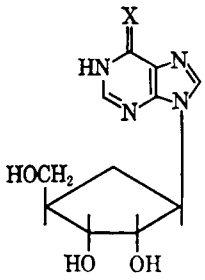
[2] (±)-*trans*-3-(6-Amino-9*H*-purin-9-yl)-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol. For the sake of brevity, the term C-Ado was employed in reports of biochemical studies (5, 6). In earlier publications (1, 2), C-Ado was designated (±)- or DL-9-[β-(2α,3α-dihydroxy-4β-(hydroxymethyl)cyclopentyl)] adenine.



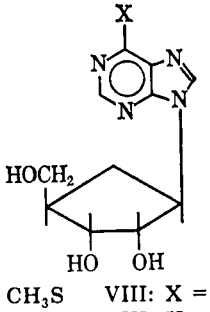
I



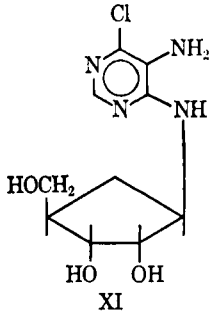
II



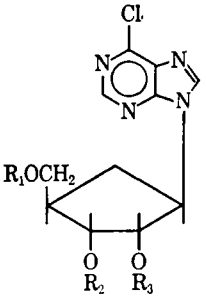
III: X = O
IV: X = S



V: X = CH_3S
VI: X = Cl
VII: X = CH_3NH
VIII: X = $(CH_3)_2N$
IX: X = HONH
X: X = CH_3O

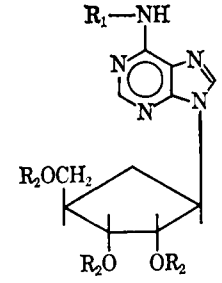


XI

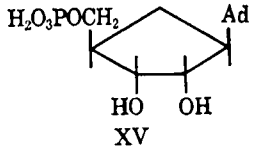


XIIa: R_1 = CHO, R_2 + R_3 = 
XIIb: R_1 = H, R_2 + R_3 =
XIIc: R_1 + R_2 + R_3 = H + 2CHO
XIId: R_1 + R_2 + R_3 = 2H + CHO

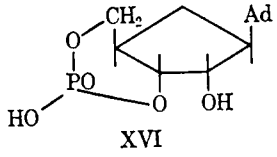
workers (5, 6) showed that II can serve as a substrate for adenosine kinase and for adenosine deaminase and as an inhibitor, presumably after it is phosphorylated, of an early step in the biosynthesis *de novo* of inosinic acid. Hill *et al.* (7) found that II-phosphate (XV), the preparation of which is reported here, is a potent inhibitor of guanylic acid kinase. Furthermore, an analog of vitamin B_{12} coenzyme prepared from II can act as a coenzyme in the reaction catalyzed by dioldehydrase (8). Of added interest are the findings that II is a moderate inhibitor of the protozoan *Tetrahymena pyriformis* (9) and that aristeromycin (10), an antibiotic for certain plant pathogens, is one enantiomeric form of II (11, 12). These biochemical and biological studies show that alteration of the nucleoside structure by replacement of the furanose oxygen atom with a methylene group can produce biologically active compounds. The preparation of carbocyclic analogs of other bio-



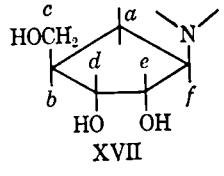
XIII: R_1 = R_2 = C_3H_7CO
XIV: R_1 = H, R_2 = C_3H_7CO



XV



XVI



XVII

chemically significant or biologically active 6-substituted purine ribonucleosides and ribonucleotides is now described.

CHEMISTRY

(±)-*trans*-3-(5-Amino-6-chloropyrimidin-4-yl)-*trans*-(5-hydroxymethyl)-*cis*-1,2-cyclopentanediol (XI) is the immediate precursor of the purine ring of carbocyclic analogs of 6-substituted purine ribonucleosides (1, 2). The pyrimidine (XI) was treated with triethyl orthoformate and concentrated hydrochloric acid to form the 6-chloropurine ring (VI), and the crude syrup obtained from the acid-catalyzed reaction was converted with anhydrous hydrogen chloride to a solid consisting of a mixture of hydrochlorides of VI and its derivatives (2). Such crude 6-chloropurine hydrochloride preparations, used previously (2) for the preparation of II and the inosine carbocyclic analog (III), furnished the 6-(methylamino)-purine (VII) and the 6-(dimethylamino)purine (VIII) derivatives by nucleophilic displacement of the 6-chloro substituent.

However, acid-catalyzed reactions of ribonucleosides with *ortho*-esters produce 2',3'-alkoxymethylene derivatives (13–16), which may be hydrolyzed under mildly acidic conditions to formate esters (14–16). Mass spectral studies of crude VI-hydrochloride specimens showed, as expected, the presence of derivatives of these types in addition to VI. Strongly acidic (and subsequent basic) conditions employed during the isolation and purification of II, VII, and VIII and during the preparation of the inosine analog (III) ensured liberation of the cyclopentyl hydroxyl groups. However, strongly acidic or basic conditions are detrimental to some 6-substituted purines, including 9-substituted 6-chloropurines. For this reason and because VI was desired as a target compound for biological evaluation, the preparation and purification of VI were investigated further. By omitting hydrochloride formation after the reaction of XI with triethyl orthoformate and by treating the reaction product successively with aqueous acetic acid and methanolic ammonia, pure VI was obtained. Mass spectral analyses at each stage indicated that: (*a*) the initial reaction product obtained from XI and triethyl orthoformate contained predominantly the formylethoxymethylene derivative (XII*a*), together with small amounts of XII*b* (or XII*c*) and VI; (*b*) the product after the aqueous acetic acid treatment consisted principally of a mixture of VI, monoformate esters (XII*d*), and diformate esters (XII*c*); and (*c*) the product after methanolic ammonia treatment was almost pure VI. TLC or the mass spectrum indicated that small amounts of II, III, X, and the 6-ethoxypurine analogous to X were formed by the sequence of treatments that began with XI. Pure VI could be obtained by recrystallization.

The 6-(hydroxyamino)purine derivative (IX) was obtained by replacement of the chloro group of purified specimens of VI with hydroxylamine by a procedure similar to that used for the ribonucleoside (17). The 6-methoxypurine (X) was prepared by treating pure VI with sodium methoxide in methanol. Previously (2), the 6-(methylthio)purine derivative (V) had been prepared by methyla-

Table I—Evaluation of Carbocyclic Analogs of 6-Substituted Purine Ribonucleosides against Neoplastic Cells *In Vitro* and *In Vivo*

Compound X	Number	H.Ep.-2[a] or KB Cells, ED_{50} (mcg./ml.)	Leukemia L-1210[b] Single-Dose Treatment[c] Dose, mg./kg.	Average Weight Change, *T/C*	*T/C*, %	*q.d.* 1–9 Dose, mg./kg./Day	Average Weight Change, *T/C*	*T/C*, %
Cl	VI	3	400	+3.7/+3.4	96	200	−2.7/+2.9[d]	93
						150	−1.4/+1.3	92
						100	−0.3/+1.3	101
						75	+0.6/+1.3	107
NH_2	II	0.7[e]	400[f]	−4.7/+1[d]	88	50	−2.1/+1.5	95
			200	−3.2/+1[d]	91	50	−1.5/+0.9	82[d]
			100	−0.4/+1	102	25	−0.4/+0.9	89
			50	+1.5/+1	93	12.5	+0.7/+0.9	97
						6.3	+1.0/+0.9	98
CH_3NH	VII	4.5	400	+1.4/+1.7	100	400	−3.7/+1.6[d]	76[d]
						200	−2.6/+1.3	84[d]
						100	−0.3/+1.3	100
$(CH_3)_2N$	VIII	>100	400	+1.5/+1.6	94			
HONH	IX	9	400	−4.6/+2.1[d]	72[d]	100	−2.9/+2.9[d]	89
			200	+0.9/+1.8	100	75	−2.1/+1.3	86
						50	−1.5/+1.3	92
						25	+0.5/+1.3	103
CH_3O	X	20 (KB)	400	+0.3/+1.4	98	200	−2.2/+2.9[d]	84[d]
						100	−1.3/+0.2	91
						50	−1.5/+0.2	94
						25	−0.3/+0.2	102
CH_3S	V	92	400	+3.0/+2.8	91	200	+0.5/+1.3	101
O	III	>100	400[f]	+2.3/+1.9	97	400	−2.4/+1.6[d]	68[d]
			200	+2.1/+1.9	92	200	0/+1.0	87
			100	+2.9/+1.9	101			
S	IV	>100	400[f]		t[g]	200	−0.2/+1.1	101
			200	+1.5/+1.0	96			
			100	+1.6/+1.0	96			
			50	+1.7/+1.0	96			
NH_2, phosphate	XV	0.7	400	−3.3/+2.0[d]	69[d]	150[h]	+1.8/+2.5	100
			300	−2.9/+1.9[d]	79[d]	75[h]	+2.2/+2.5	110
			150	−0.7/+1.9	87	37[h]	+1.4/+2.5	105
			75	+1.5/+1.9	107			
NH_2, cyclic phosphate	XVI	3.6[i]				50	+0.7/+1.8	115
						25	+1.5/+0.4	103

[a] ED_{50} determined for H.Ep.-2 cells unless otherwise indicated. H.Ep.-2 = human epidermoid carcinoma cells, No. 2, growing in cell culture. KB = Eagle's KB cells growing in cell culture and derived from a carcinoma of the human nasopharynx. ED_{50} = concentration of a compound that inhibits cell growth, measured by protein determinations, to 50% of the growth of untreated cells. [b] Mice were implanted intraperitoneally on Day 0 with 10^6 L-1210 cells. For single-dose treatment, a compound was injected intraperitoneally on Day 1 (about 24 hr.) or on Day 2 (about 48 hr.) after implantation; *q.d.* 1–9 means that daily injections at the specified dose were initiated on Day 1 and continued through Day 9 or until the death of the animal. Average weight change = average weight change of host animals. Weight changes were determined 4 days after the first (for daily treatment) or only injection. *T* = treated mice; *C* = control mice. [c] Treatment on Day 1 unless otherwise indicated. [d] $T/C < 85\%$ and a difference in weight change ($T-C$) greater than 4 g. are criteria of toxicity to the host animals. [e] Approximate average of several tests. ED_{50} determined by the clone-colony method = 0.7 μmole/ml. (6). [f] Single-dose treatment with this compound was performed on Day 2. [g] Half of the treated animals died on or before Day 5. [h] The treatment schedule was Days 1, 5, and 9 rather than *q.d.* 1–9. [i] Average of three tests.

tion of the purine-6-thione (IV). The availability of pure VI permitted the synthesis of V by methylmercaptide ion generated from sodium methoxide and hydrogen sulfide in methanol (18). Proton magnetic resonance analysis of recrystallized V showed that it contained no more than 1% of the 6-methoxypurine (X). The 6-(methylamino)purine (VII) and the adenine (II) derivatives were also obtained more conveniently and in superior yields from purified VI.

The following derivatives of II were synthesized: the tetrabutyryl (XIII) and tributyryl (XIV) derivatives; the 5-dihydrogen phosphate (XV), which is the (±)-analog of adenylic acid; and the cyclic 1,5-phosphate (XVI), which is the (±)-analog of cyclic 3′,5′-adenosine monophosphate. The phosphate (XV) was obtained in 67% yield by phosphorylating isopropylidene II with phosphorus oxychloride in trimethyl phosphate (19, 20), isolating the product as the ammonium salt, and converting the salt to the free acid. After this work had been completed, the preparation of aristeromycin phosphate was reported by Imai *et al.* (21). The cyclic phosphate (XVI) was synthesized from XV by the method of Smith *et al.* (22) for the preparation of cyclic adenosine monophosphate; a simplified isolation procedure afforded a good yield of XVI.

BIOLOGICAL EVALUATION

Most purine ribonucleosides are not active *per se;* observed biological effects are caused by ribonucleotides formed from the ribonucleosides (23). Since it is generally accepted that nucleotides do not penetrate cell walls, activity is dependent on intracellular activation by kinases or by the sequential action of phosphorylases and phosphoribosyl transferases (23). Some new derivatives (VI–VIII and X) were selected, therefore, because the analogous ribonucleosides are good substrates for adenosine kinase from human epidermoid carcinoma (H.Ep.-2) cells (24), and these derivatives might also be phosphorylated by this enzyme. Additionally, the 6-(hydroxyamino)purine derivative (IX) is the analog of a nucleoside having anticancer activity (17, 25), and the cyclic phosphate (XVI) is the analog of cyclic adenosine monophosphate which may, in some degree, be transported intact into cells (26, 27).

The adenine derivative (II) is a highly cytotoxic compound (5, 6). The 6-chloropurine (VI), 6-(methylamino)purine (VII), and 6-(hydroxyamino)purine (IX) derivatives and the two nucleotide analogs (XV and XVI) also proved to be cytotoxic to neoplastic cells growing in culture (Table I), and the 6-methoxypurine (X) was modestly inhibitory. There is no evidence to indicate whether XV and XVI are cytotoxic as such or whether the observed activity is due to cleavage to II. As mentioned in the introduction, XV was found to be a potent inhibitor of guanylic acid kinase (7).

The results shown in Table I summarize tests of carbocyclic analogs of 6-substituted purine ribonucleosides against leukemia L-1210 in mice. All of the nucleoside analogs (II–X) were tested on a single-dose schedule (Day 1 or 2) at 400 mg./kg. If toxic symptoms appeared at this dose, testing was continued at lower doses. The 6-chloropurine (VI), 6-aminopurine (II), 6-(methylamino)purine (VII), 6-(hydroxyamino)purine (IX), and 6-methoxypurine (X) derivatives and the hypoxanthine (III) were administered at several

doses on a *q.d.* 1–9 schedule. These doses ranged from an upper, toxic dose (indicated by host weight loss or T/C <85%) to lower doses that caused little, or no, weight loss. The remaining compounds shown in Table I received more limited testing *in vivo*. None of the tests performed gave evidence of activity. The results in Table I indicate the degree of toxicity of these compounds *in vivo* (to leukemia-bearing mice) and show the scope of the evaluation.

EXPERIMENTAL[3]

(±)-*trans*-3-(6-Chloro-9*H*-purin-9-yl)-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol (VI)—A solution of 500 mg. of XI, 15 ml. of triethyl orthoformate, and 0.15 ml. of 12 *N* hydrochloric acid was stirred at room temperature for 19 hr. The solution was concentrated *in vacuo* at 25° to a syrup, and several portions of toluene were similarly evaporated from the residue to aid in the removal of traces of volatile components. The mass spectrum (direct-probe inlet temperature 395°) of the residue indicated that it was composed principally of derivatives of VI formed by the reaction of triethyl orthoformate with the hydroxyl groups, along with trace amounts of VI and other components[4,5]: *m/e* 368 (M^+ of XII*a*, weak), 340 (M^+ of XII*b* or XII*c*, very weak), 339 (XII*a* − CHO), 323 (XII*a* − OC_2H_5, very strong), 295 (XII*b* − OC_2H_5 or 323 − CO), 284 (M^+ of VI, very weak), 235 (XII*a* − $OCHOC_2H_5$ − CH_2OCHO, very strong), 155 (P + 2H), 154 (P + H), and 153 (P); relative peak heights above 150 mass units, *m/e* 155 > 323 > 235 > 153. The fact that the peak of *m/e* 323 was the second strongest in the spectrum indicates that the crude product was mostly XII*a*; the strong peak at *m/e* 235 is consistent with this interpretation, but it also might have arisen partly from XII*b* (340 − $OCHOC_2H_5$ − CH_2OH).

To hydrolyze the ethoxymethylene group of XII*a* and XII*b* and other *ortho*-ester groups that might be present, a solution of the crude product in 10 ml. of 50% acetic acid was stirred at room temperature for 5.5 hr. and then concentrated to a syrup. To remove residual acetic acid, a solution of the syrup in water was lyophilized, yielding 553 mg. An IR spectrum showed strong ester absorption at 1710 $cm.^{-1}$ (—OCHO), and the mass spectrum[4,5] (direct-probe inlet temperature 390°) indicated that the product was chiefly a mixture of VI and its formate esters: *m/e* 340 (M^+ of XII*c*), 312 (M^+ of XII*d*, stronger than 284 and 340), 311 (XII*c* − CHO), 294 (XII*d* − H_2O), 284 (M^+ of VI), 283 (XII*d* − CHO), 253 (VI − CH_2OH or XII*d* − CH_2OCHO), 235 (XII*d* − CH_2OCHO − H_2O or VI − CH_2OH − H_2O), 181 (P + C_2H_4), 155 (P + 2H, base peak), 154 (P + H), and 153 (P); relative peak heights above 100 mass units, 155 > 157 > 181 > 154 ~ 235 > 156 > 119 > 183. The peak of *m/e* 235 must have been derived chiefly from XII*d*, because it was much stronger than the molecular ion peak of VI and it was much weaker than *m/e* 284 in the spectra of pure specimens of VI. Weak peaks of *m/e* 368 (XII*a*), 358, 330, 323 (XII*a* − OC_2H_5), and 302 showed the presence of other minor components.

A solution of 353 mg. of the gummy solid in 10 ml. of 10% ammonia–methanol was kept at room temperature for 4 hr. to remove the formyl groups. After evaporation of the solvent and of several portions of ethanol from the reaction product, it was stirred with a mixture of ethyl acetate (7 ml.) and ethanol (3 ml.) until solidification occurred, yielding 227 mg. (68% yield from XI), m.p. 152–170° dec. UV data and the mass spectrum (compared with spectra of pure specimens of VI) indicated that this material was almost pure VI; TLC showed that it was VI contaminated with small amounts (less than 3–5%) of both II and the hypoxanthine derivative (III). These impurities must have been introduced by displacement of the 6-chloro group by ammonia and *via* acidic hydrolysis, respectively. Also, weak peaks of *m/e* 280 and 294 in the mass spectrum correspond to the molecular ions of the 6-methoxypurine (X) and the analogous 6-ethoxypurine derivative, respectively, and suggest that slight displacement of the 6-chloro group by methanol and ethanol occurred. Recrystallization of such specimens from a small volume of ethanol–ethyl acetate or from ethanol–hexane (1:1) gave pure VI, m.p. 171–175° dec.; TLC, 1 spot [20 mcg., chloroform–methanol (4:1)]; UV: λ_{max} 265 (ϵ 9400) at pH 1 and 7; mass spectrum[4,5] (direct-probe inlet temperature 390°): *m/e* 284 (M^+), 267 (M − OH), 266 (M − H_2O), 256 (M − CO), 255, 253 (M − CH_2OH), 237, 235 (M − CH_2OH − H_2O), 225 (M − CH_2OH − CO), 219, 209, 197, 181 (P + C_2H_4), 167, 155 (P + 2H), 154 (P + H), and 153 (P). The peak of *m/e* 155 was the base peak in the spectra of purified specimens of VI. The peaks of *m/e* 157 (due to the ^{37}Cl-fragment analogous to *m/e* 155) and *m/e* 181 were the next strongest; other strong peaks above 100 mass units were 156, 154, 183, 119, 284, and 209.

Anal.—Calc. for $C_{11}H_{13}ClN_4O_3$: C, 46.40; H, 4.60; Cl, 12.45; N, 19.75. Found: C, 46.35; H, 4.69; Cl, 12.72; N, 19.51.

Crude 6-chloropurine (VI) hydrochloride specimens were used for preparing some 6-substituted purine derivatives. These very hygroscopic, white specimens were prepared by the previously described procedure (2) from XI, triethyl orthoformate, and concentrated hydrochloric acid, followed by treatment of the crude syrup with hydrogen chloride to obtain a solid product. Several specimens of the crude mixture of hydrochlorides gave similar (weak) mass spectral patterns. Dissolution of such specimens in water to dissociate the hydrochloride salts, followed by lyophilization, increased the volatility of the crude products; mass spectra were improved but not appreciably changed. Typical mass spectra showed peaks corresponding to the molecular ions of VI, XII*b* or XII*c*, XII*d*, and fragments derived from these compounds. Since the crude hydrochloride preparations are very hygroscopic, hydrolysis of some ethoxymethylene and formyl groups may have occurred during handling. Weak molecular ion peaks of *m/e* 358, 348 and 330, and 302 in the spectra correspond to by-products in which one or two hydroxyl groups of XII*b* or XII*c*, XII*d*, and VI, respectively, are replaced by chloro groups. These minor by-products may have been introduced during the preparation of the crude mixture of hydrochlorides or during one of the two preceding steps (2) and, because of their greater volatility, may have been detectable in trace amounts.

(±)-*trans*-3-(Hydroxymethyl)-*trans*-5-[6-(methylamino)-9*H*-purin-9-yl]-*cis*-1,2-cyclopentanediol (VII)—A solution of 1.0 g. of crude VI hydrochloride (2) and 60 ml. of liquid methylamine was heated in a glass-lined, stainless steel bomb at 70° for 18 hr. and then concentrated to a syrup. A solution of the syrup, 30 ml. of water, 30 ml. of ethanol, and 3 ml. of 12 *N* HCl was heated at 45° for 0.5 hr. The solvents were evaporated *in vacuo*, and several portions of water were evaporated from the residue. A solution of the residue in 150 ml. of water was stirred with 10 g. of an anion-exchange resin[6] for 0.5 hr., the resin was removed by filtration and washed several times with 50-ml. portions of warm water, and the residue remaining after evaporation *in vacuo* of the combined filtrate and wash solutions was dried further by evaporating several portions of ethanol from it. A solution of the amorphous residue in 50 ml. of ethanol deposited 693 mg. (80%) of crude VII free base (m.p. 103–106° dec.). A solution of 670 mg. of the crude free base, 25 ml. of ethanol, and 15 ml. of ether was treated with anhydrous hydrogen chloride. A white precipitate, with the approximate composition of a dihydrochloride, was recrystallized from methanol–ether, yielding 555 mg. of the monohydrochloride of VII, m.p. 177–

[3] UV spectra were recorded with a Cary model 17 or 14 spectrophotometer. UV maxima are in nanometers; sh = shoulder. Solutions for UV determinations were prepared by diluting a 5-ml. aliquot of a water or ethanol solution of the compound to 50 ml. with 0.1 *N* HCl, phosphate buffer (pH 7), or 0.1 *N* NaOH; absorption maxima are reported at pH 1, 7, and 13. IR spectra were recorded with a Perkin-Elmer model 621 or 521 spectrometer from samples in KBr disks. Mass spectral data were taken from low-resolution spectra determined with a Hitachi-Perkin-Elmer RMU-7 double-focusing instrument; the peaks listed are those due to the molecular ion (M^+), those attributable to the loss of certain fragments from the molecular ion (M − a fragment), and other prominent peaks due to fragments heavier than the purine moiety (minus the cyclopentyl group). Proton magnetic resonance (PMR) spectra were determined with a Varian model XL-100-15 spectrometer for observing proton resonance at 100 MHz. Chemical shift data (δ) are in parts per million downfield from tetramethylsilane, the internal reference; s = singlet, and m = multiplet. Melting temperatures were determined in capillary tubes heated in a Mel-Temp apparatus. Unless otherwise indicated, TLC was performed on plates of silica gel, and spots were detected by UV light (254 nm.) after spraying the chromatogram with an optical whitening agent (Ultraphor WT, BASF Colors and Chemicals, Inc., Charlotte, N. C.) and by spraying with a basic solution of potassium permanganate. The quantity applied and the developing solvent are shown parenthetically at the appropriate places in the procedures.

[4] The chlorine-containing fragments listed contain ^{35}Cl; the corresponding fragments (heavier by 2 mass units) containing ^{37}Cl are not listed unless the peaks were stronger than expected from the isotope ratio.

[5] P = the purine ring fragment without a 9-substituent.

[6] Dowex 1-X8, OH^- form.

179° dec.; UV: λ_{max} 263 (ε 17,200) at pH 1 and 267 (ε 16,800) at pH 7 and 13.

Anal.—Calc. for $C_{12}H_{17}N_5O_3 \cdot HCl$: C, 45.65; H, 5.74; N, 22.17. Found: C, 45.74; H, 5.79; N, 22.26.

Pure VI (500 mg.) was treated with 30 ml. of methylamine according to the procedure already described for crude VI hydrochloride. Concentration of the reaction mixture to dryness left a white solid. The product was recrystallized twice from ethanol and dried *in vacuo* at 78°, yielding 440 mg. (77%), m.p. 110–112° dec.; TLC, 1 spot [60 mcg., chloroform–methanol (3:1)]; mass spectrum (direct-probe inlet temperature 240°): *m/e* 279 (M^+), 262 (M − OH), 248 (M − CH_2OH), 232, 230 (M − CH_2OH − H_2O), 220 (M − CH_2OH − CO), 204, 192, 176 (P + C_2H_4), 150 (P + 2H, base peak), 149 (P + H), and 148 (P).

Anal.—Calc. for $C_{12}H_{17}N_5O_3 \cdot C_2H_5OH$: C, 51.68; H, 7.12; N, 21.53. Found: C, 51.72; H, 7.43; N, 21.36.

(±)-*trans*-3-[6-(Dimethylamino)-9*H*-purin-9-yl]-*trans*-(5-hydroxymethyl)-*cis*-1,2-cyclopentanediol (VIII)—A solution of 350 mg. of crude VI hydrochloride (2) in 12 ml. of 25% aqueous dimethylamine was heated under reflux for 3 hr., allowed to stand at room temperature for 5 days, and evaporated *in vacuo* to a syrup. By a procedure similar to that described for the preparation of VII from crude VI, the dihydrochloride of VIII was obtained from a chilled mixture of ethanol, ether, and dry hydrogen chloride, yielding 130 mg. (32%), m.p. 98–105° with premature softening; UV: λ_{max} 268 (ε 17,700) at pH 1 and 277 (ε 18,000) at pH 7 and 13.

Anal.—Calc. for $C_{13}H_{19}N_5O_3 \cdot 2HCl$: C, 42.63; H, 5.78; N, 19.12. Found: C, 42.78; H, 5.52; N, 18.84.

trans-3-[6-(Hydroxyamino)-9*H*-purin-9-yl]-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol (IX)—A solution of 854 mg. of pure VI, 6 ml. of methanol, 6 ml. of ethanol, and 2.5 g. of hydroxylamine (prepared from the hydrochloride and sodium methoxide in methanol) was heated under reflux for 3 hr. and then allowed to stand at room temperature for 18 hr. A white precipitate was separated by filtration, washed with ethanol, and dried *in vacuo* at 78°, yielding 655 mg., m.p. 204–220° dec. The crude product was dissolved in 50% aqueous ethanol, the solution was concentrated to a syrupy consistency, ethanol was added, and the solution was chilled. The first crop (357 mg.) of IX was stirred with water, washed with ethanol, and dried *in vacuo* at 78°, yielding 313 mg., m.p. 225° dec.; TLC, 1 spot [40 mcg., butanol–water–acetic acid (5:3:2)]; UV: λ_{max} 266 (ε 16,700) at pH 1 and 267 (ε 13,700) at pH 7.

Anal.—Calc. for $C_{11}H_{15}N_5O_4$: C, 46.97; H, 5.37; N, 24.90. Found: C, 46.64; H, 5.65; N, 24.89.

A second crop of IX, obtained by concentrating the filtrate and cooling it to −20°, was recrystallized from aqueous ethanol, yielding 73 mg., m.p. 224° dec.; UV: λ_{max} 266 (ε 16,500) at pH 1.

(±)-*trans*-3-(Hydroxymethyl)-*trans*-5-(6-methoxy-9*H*-purin-9-yl)-*cis*-1,2-cyclopentanediol (X)—A solution prepared from 600 mg. of pure VI, 14 ml. of anhydrous methanol (dried with molecular sieves), and 6 ml. of 1 *N* sodium methoxide in methanol was heated under reflux for 2 hr., allowed to stand at room temperature for 48 hr., and evaporated to dryness *in vacuo*. A solution of the residue in 10 ml. of water was acidified with 1 ml. of 6 *N* HCl and then neutralized with solid sodium bicarbonate. The mixture, containing a precipitate, was allowed to stand at 5° and was then filtered to separate white crystals, yielding 490 mg., m.p. 203–210° dec. The crude product was recrystallized from water and dried *in vacuo* at 78°, yielding 387 mg. (65%), m.p. 210–212° dec.; TLC, 1 spot [40 mcg., chloroform–methanol (3:1)]; UV: λ_{max} 252 (ε 10,800) and 262 (sh) at pH 1 and 250–254 (ε 11,400) and 262 (sh) at pH 7 and 13; mass spectrum[5] (direct-probe inlet temperature 250°): *m/e* 280 (M^+), 263 (M − OH), 262 (M − H_2O), 252 (M − CO), 249 (M − CH_2OH), 233, 231 (M − CH_2OH − H_2O), 221 (M − CH_2OH − CO), 205, 193, 177 (P + C_2H_4), 163, 151 (P + 2H, base peak), 150 (P + H), and 149 (P); PMR[7] (dimethyl sulfoxide-d_6): δ 1.6–about 2.5 (m, H*a*H*a*H*b*), 3.4–3.63 (m, H*c*H*c*), 3.78–3.96 (m, H*d*), 4.1 (s, CH_3O), 4.24–4.5 (m, H*e*), 4.56–4.98 (m, H*f* + 3OH), 8.44 (s, H_8 of purine ring), 8.50 (s, H_2 of purine ring), and 4.62–5.04 (after addition of D_2O, m, H*f*). A specimen for analysis was dried at 110°.

Anal.—Calc. for $C_{12}H_{16}N_4O_4$: C, 51.40; H, 5.75; N, 19.99. Found: C, 51.15; H, 5.48; N, 19.60.

(±)-*N*-{9-[*trans*-2,*trans*-3-Dihydroxy-*cis*-4-(hydroxymethyl)cyclopentyl]-9*H*-purin-6-yl}butyramide Tributyrate (XIII)—An anhydrous mixture of 500 mg. of II, 10 ml. of pyridine, and 7.5 ml. of butyric anhydride was stirred at room temperature for 2 days and then concentrated *in vacuo* to a solid residue. White crystalline XIII was obtained by triturating the residue with ethanol–hexane (1:9) and recrystallizing the crude product (821 mg.) from ethyl acetate–hexane (4:15), yielding 723 mg. (70%), m.p. 116–117°; UV: λ_{max} 282 (ε 17,800) at pH 1.

Anal.—Calc. for $C_{27}H_{39}N_5O_7$: C, 59.43; H, 7.21; N, 12.84. Found: C, 59.25; H, 7.24; N, 12.87.

(±)-*trans*-3-(6-Amino-9*H*-purin-9-yl)-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol Tributyrate (XIV) Picrate—A solution of 190 mg. of XIII, 229 mg. of picric acid, and 11 ml. of methanol was heated under reflux for 45 min. A yellow precipitate was collected by filtration, washed with methanol, and recrystallized from an ethanol–acetone mixture, yielding 180 mg. (73%), m.p. 190–195°.

Anal.—Calc. for $C_{23}H_{33}N_5O_6 \cdot C_6H_3N_3O_7$: C, 49.43; H, 5.17; N, 15.92. Found: C, 49.25; H, 5.03; N, 15.96.

(±)-*trans*-3-(6-Amino-9*H*-purin-9-yl)-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol 5-Dihydrogen Phosphate (XV)—A solution of 12 ml. of trimethyl phosphate (19, 20), 0.732 ml. (8 mmoles) of phosphorus oxychloride, and 1.188 g. (3.9 mmoles) of the isopropylidene derivative (2) of II was prepared at −10°, stirred for 3 hr. at from −5 to −10°, and poured into 350 ml. of water. The resulting solution was allowed to stand at 25° for 1 hr., neutralized with 2.5 *N* aqueous ammonia, and passed through a column of a cation-exchange resin[8] (20 g.). The column was washed with 300 ml. of water and then with 400 ml. of 1 *N* aqueous ammonia. The ammonia effluent was concentrated *in vacuo* to a low volume (about 10 ml.), and ethanol (100 ml.) was added and the evaporation continued. Then additional ethanol (100 ml.) was added, and a white precipitate was collected by filtration and dried *in vacuo* over phosphorus pentoxide, yielding 1.036 g. (70%, calculated as a monohydrate of the ammonium salt of XV[9]). A solution of 1 g. of this material in 2 ml. of water was neutralized with 0.5 ml. of 6 *N* hydrochloric acid, diluted with 5 ml. of ethanol, and cooled to −20°. The white precipitate was dried *in vacuo* at 78° over phosphorus pentoxide, yielding 901 mg. (adjusted yield 67%), m.p. 250–252° dec.; TLC, 1 spot [60 mcg. on cellulose, butanol–water–acetic acid (5:3:2), detection by UV light after spraying[10]]. A specimen was recrystallized from water–ethanol and dried as before, m.p. 251–253° dec.; UV: λ_{max} 258 (ε 14,500) and 212 (ε 21,000) at pH 1 and 261 (ε 15,100) and 204 (ε 21,900) at pH 7.

Anal.—Calc. for $C_{11}H_{16}N_5O_6P \cdot {}^1/_2H_2O$: C, 37.29; H, 4.84; N, 19.77; P, 8.74. Found: C, 37.43; H, 4.60; N, 19.56; P, 8.64.

(±)-*trans*-3-(6-Amino-9*H*-purin-9-yl)-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol Cyclic 1,5-Hydrogen Phosphate (XVI)—A solution of 353 mg. of II-phosphate (XV), 293 mg. of 4-morpholino-*N*,*N*′-dicyclohexylcarboxamidine, 25 ml. of pyridine, and 5 ml. of water was evaporated to dryness *in vacuo*, and three 15-ml. portions of pyridine were added and evaporated separately *in vacuo* to remove water. A solution of the residue in 100 ml. of anhydrous pyridine was added dropwise during 3 hr. to a refluxing solution of 412 mg. of dicyclohexylcarbodiimide in 100 ml. of anhydrous pyridine, and the mixture was heated under reflux for an additional hour and then evaporated to dryness *in vacuo*. A mixture of the residue and water (250 ml.) was allowed to stand at room temperature for about 2 hr. and then concentrated *in vacuo* to dryness. Equal volumes of water and ether (100 ml.) were added, the mixture was shaken and filtered to remove dicyclohexylurea (m.p. 222–227°), and the aqueous layer was evaporated *in vacuo* to a yellow syrup. A solution of the syrup in 25 ml. of water was stirred with 3 g. of a strongly basic ion-exchange resin[11] for 15 min., the resin was separated by filtration and washed with water (10 × 3 ml.), and the product was recovered by stirring the resin with 175 ml. of 25% acetic acid. The aqueous acetic acid filtrate was concentrated *in vacuo* to a small volume (10 ml.), diluted with ethanol (10 ml.), chilled, and filtered to remove the white precipitate which was dried *in vacuo* at 78°, yielding 259 mg. (79%). The cyclic phosphate (XVI) was recrystallized from water (40 ml.) and dried *in vacuo* at 110°, yielding 198 mg. (61%), m.p. 268–272° dec.; UV: λ_{max} 258

[7] The positions of the protons are shown in Structure XVII.

[8] Amberlite CG-120, H^+ form.

[9] Analytical data from a similar product of another experiment were in approximate agreement with the monoammonium salt monohydrate.

[10] With Ultraphor.

[11] Rexyn 201, OH^- form.

(ε 13,500) and 211 (ε 20,600) at pH 1 and 261 (ε 14,100) and 205 (ε 20,400) at pH 7; TLC [silica gel, detection by UV and spraying[10], propanol–water (3:1)], XVI moved similarly to II and faster than XV; TLC [butanol–water–acetic acid (5:3:2)], XVI moved slower than II and slightly faster than XV.

Anal.—Calc. for $C_{11}H_{14}N_5O_5P$: C, 40.37; H, 4.31; N, 21.44; P, 9.46. Found: C, 39.98; H, 4.47; N, 21.71; P, 9.37.

(±)-*trans*-3-(6-Amino-9*H*-purin-9-yl)-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol (II)—This compound was obtained in better yield and by a simpler procedure by beginning with purified VI (*cf.* 2). A mixture of 1.215 g. of VI and 20 ml. of liquid ammonia was heated in a glass-lined, stainless steel bomb at 60° for 20 hr. and then at 80° for 1 hr. The residue obtained by evaporating the ammonia with a current of nitrogen was stirred with 20 ml. of warm 90% ethanol. Compound II (885 mg., m.p. 238–247° dec.) was separated by filtration, recrystallized from water (35 ml.), and washed with ethanol, yielding 768 mg. (white crystals), m.p. 245–247° dec. A second crop (113 mg., m.p. 231–240° dec.) of II from the 90% ethanol filtrate was recrystallized from water, yielding 86 mg., m.p. 244–248° dec. TLC and UV data showed that both portions (total yield 75%) were pure II.

(±)-*trans*-3-(Hydroxymethyl)-*trans*-5-[6-(methylthio)-9*H*-purin-9-yl]-*cis*-1,2-cyclopentanediol (V)—This compound was prepared by an improved procedure (*cf.* 2). A solution consisting of 780 mg. of purified VI, 30 ml. of anhydrous methanol (dried with molecular sieves), and 5.5 ml. of a 1 *N* methanol solution of sodium methoxide was saturated with methanethiol at 5°. The solution was heated at 100° in a glass-lined, stainless steel bomb for 18 hr. and then concentrated to dryness *in vacuo*. A water solution (25 ml.) of the residue was acidified to pH 3 with 1 *N* HCl, neutralized by the addition of solid sodium bicarbonate[12], and evaporated to dryness *in vacuo*. The residual solid was leached repeatedly (10 times) with a boiling ethyl acetate–acetone (4:1) mixture. The total extract was concentrated *in vacuo* to about 10 ml., and the precipitate (630 mg.) was separated by filtration. Recrystallization of the crude product from ethanol gave 605 mg. (74%) of white crystals, m.p. 179–181° dec.; mass spectrum (direct-probe inlet temperature 270°): *m/e* 296 (M^+), 279 (M − OH), 278 (M − H_2O), 265 (M − CH_2OH), 249, 247 (M − CH_2OH − H_2O), 237 (M − CH_2OH − CO), 231, 222, 221, 209, 193 (P + C_2H_4), 179, 167 (P + 2H, base peak), 166 (P + H), and 165 (P); PMR[7] (dimethyl sulfoxide-d_6): δ 1.64–about 2.5 (m, H*a*H*a*H*b*), 2.68 (s, CH_3S), 3.42–3.64 (m, H*c*H*c*), 3.8–3.96 (m, H*d*), 4.24–4.52 (m, H*e*), 4.58–5.06 (m, H*f* + 3OH), 8.53 (s, H_8 of purine ring), 8.7 (s, H_2 of purine ring), and 4.66–5.06 (after addition of D_2O, m, H*f*). A very weak singlet at 4.1 p.p.m. indicated that this sample contained about 1% of the 6-methoxypurine derivative (X).

REFERENCES

(1) Y. F. Shealy and J. D. Clayton, *J. Amer. Chem. Soc.*, **88**, 3885 (1966).

(2) *Ibid.*, **91**, 3075(1969).

(3) Y. F. Shealy and C. A. O'Dell, *Tetrahedron Lett.*, **1969**, 2231.

(4) K. C. Murdock and R. B. Angier, *J. Amer. Chem. Soc.*, **84**, 3758(1962).

(5) P. W. Allan, D. L. Hill, and L. L. Bennett, Jr., *Fed. Proc.*, **26**, 730(1967).

(6) L. L. Bennett, Jr., P. W. Allan, and D. L. Hill, *Mol. Pharmacol.*, **4**, 208(1968).

(7) D. L. Hill, S. Straight, P. W. Allan, and L. L. Bennett, Jr., *ibid.*, **7**, 375(1971).

(8) S. S. Kerwar, T. A. Smith, and R. H. Abeles, *J. Biol. Chem.*, **245**, 1169(1970).

(9) D. L. Hill, S. Straight, and P. W. Allan, *J. Protozool.*, **17**, 619(1970).

(10) T. Kusaka, H. Yamamoto, M. Shibata, M. Muroi, T. Kishi, and K. Mizuno, *J. Antibiot.*, **21**, 255(1968).

(11) T. Kishi, M. Muroi, T. Kusaka, M. Nishikawa, K. Kamiya, and K. Mizuno, *Chem. Commun.*, **1967**, 852.

(12) T. Kishi, M. Muroi, T. Kusaka, M. Nishikawa, K. Kamiya, and K. Mizuno, *Chem. Pharm. Bull.*, **20**, 940(1972).

(13) J. Žemlička, *Chem. Ind.*, **1964**, 581.

(14) M. Jarman and C. B. Reese, *ibid.*, **1964**, 1493.

(15) C. B. Reese and J. E. Sulston, *Proc. Chem. Soc.*, **1964**, 214.

(16) F. Eckstein and F. Cramer, *Chem. Ber.*, **98**, 995(1965).

(17) A. Giner-Sorolla, L. Medrek, and A. Bendich, *J. Med. Chem.*, **9**, 143(1966).

(18) J. A. Montgomery and K. Hewson, *ibid.*, **9**, 354(1966).

(19) M. Yoshikawa, T. Kato, and T. Takenishi, *Tetrahedron Lett.*, **1967**, 5065.

(20) A. Yamazaki, I. Kumashiro, and T. Takenishi, *Chem. Pharm. Bull.*, **16**, 338(1968).

(21) K.-I. Imai, S. Fujii, K. Takanohashi, Y. Furukawa, T. Masuda, and M. Honjo, *J. Org. Chem.*, **34**, 1547(1969).

(22) M. Smith, G. I. Drummond, and H. G. Khorana, *J. Amer. Chem. Soc.*, **83**, 698(1961).

(23) J. A. Montgomery, in "Progress in Medicinal Chemistry," vol. 7, part 1, G. P. Ellis and G. B. West, Eds., Butterworth and Co., Ltd., London, England, 1970, chap. 2.

(24) H. P. Schnebli, D. L. Hill, and L. L. Bennett, Jr., *J. Biol. Chem.*, **242**, 1997(1967).

(25) J. H. Burchenal, M. Dollinger, J. Butterbaugh, D. Stoll, and A. Giner-Sorolla, *Biochem. Pharmacol.*, **16**, 423(1967).

(26) R. A. Levine, S. E. Lewis, J. Shulman, and A. Washington, *J. Biol. Chem.*, **244**, 4017(1969).

(27) M. L. Heidrick and W. L. Ryan, *Biochim. Biophys. Acta*, **237**, 301(1971).

[12] If the solution is not neutralized after acidic decomposition of the excess sodium methylmercaptide, residual acid may catalyze the reaction of V with the leaching solvents; mass spectra of the products of earlier experiments in which sodium bicarbonate was not added prior to evaporation of solvents showed the presence of small amounts of a monoacetate of V (formed by transesterification) and the isopropylidene derivative of V.

ACKNOWLEDGMENTS AND ADDRESSES

Received December 26, 1972, from the *Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, AL 35205*

Accepted for publication March 15, 1973.

Supported by Contracts NIH-71-2021 and PH43-64-51 with the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health. Testing against L-1210 leukemia, under the supervision of Dr. F. M. Schabel, Jr., and Dr. W. R. Laster, Jr., and cytotoxicity tests, under the supervision of Dr. L. J. Wilkoff and Dr. G. J. Dixon, were supported by Contracts PH43-65-594 and NIH-71-2098 with the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health.

Elemental analyses and spectrometric determinations were performed under the supervision of Dr. W. C. Coburn, Jr., by members of the Molecular Spectroscopy Section of this Institute. Mass spectra were determined and interpreted by Mr. Marion Kirk; PMR spectra were determined and interpreted by Mrs. Martha Thorpe. Some elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

▲ To whom inquiries should be directed.