drogenation of 15 proceeded cleanly only with selenium dioxide in neutral, strictly anhydrous *tert*-butyl alcohol to give 12.[10] Compound 13 was prepared by reaction of 17-acetyl-19-nortestosterone with iodine–potassium iodate,[11] followed by selenium dioxide dehydrogenation as above. Alternatively, epoxidation of the known alcohol 16 with *m*-chloroperbenzoic acid gave 17, although in minor amount compared to the isomeric β-epoxides.[12] Chromium trioxide–pyridine oxidation of 17, followed by selenium dioxide dehydrogenation, gave 13.[13]

Dehydrogenation of 17β-hydroxy-19-nor-5α-androstan-3-one was accomplished by sequential treatment with phenylselenyl chloride in ethyl acetate and hydrogen peroxide. Elimination of phenylselenenic acid at room temperature gave the 1-en-3-one, which with alkaline hydrogen peroxide gave one of the 1,2-epoxy-3-ones, 18, stereospecifically. Reduction of the 1-en-3-one with sodium borohydride gave both stereoisomers of 19. These were epoxidized with *m*-chloroperbenzoic acid and subsequently oxidized with chromium trioxide–pyridine to generate both stereoisomers of 18. The stereochemistry of neither isomer of 18 could be assigned unequivocally from the ^{1}H NMR spectra; X-ray work will be utilized to settle these points. The more abundant isomer resulting from these experiments has been dehydrogenated with selenium dioxide in *tert*-butyl alcohol to give 14.[14]

The three epoxyenones thus obtained are similar and remarkably stable. They do undergo smooth aromatization under acidic conditions; 12 and 13 form the triacetate of 2 by the action of *p*-toluenesulfonic acid in acetic anhydride, and 14 yields, under similar conditions, the known triacetate of 3.[8] On the other hand, the epoxyenones may be recovered unchanged from methanolic sodium methoxide (20 °C, 12 h) or potassium *tert*-butoxide–*tert*-butyl alcohol (20 °C, 12 h).

The synthesis of these tautomers of dienol epoxides derived from estradiol and their smooth aromatization suggest that they, in equilibrium with their dienol tautomers, may be intermediates in catechol estrogen biosynthesis. The aromatization step may be simply acid catalyzed or may be enzyme mediated.

Compounds 12–14 have been biologically evaluated in two ways. First, their activity on bacteriophage PM2 DNA, as measured by the creation of endonuclease-sensitive sites and subsequent agarose gel electrophoresis, was evaluated.[15] The compounds were inactive by these criteria. Secondly, the compounds were subjected to the foci assay of Kakunaga,[16] which involves determination of the chemical transformation of Balb/c 3T3 mouse fibroblast cells. From the 1-1A-10 line obtained from Dr. Takeo Kakunaga, a subclone (MN-20) was isolated which showed good contact inhibition, flat surface morphology, low spontaneous transformation frequency, and reproducible transformation by 3-methylcholanthrene. Initial findings indicate, interestingly, that in this assay compound 13 (2 μM) is approximately as active as the highly mutagenic 3-methylcholanthrene itself in inducing chemical transformation of the cells and at least two orders of magnitude more effective than estradiol. Compounds 12 and 14 were inactive at the same concentration as used for compound 13. Extensions of these chemical and biological experiments are in progress.

Acknowledgment. The work at Northeastern University was supported by the Office of Naval Research, Department of the Navy (Contract N00014-76-C-0027). The work at the Southwest Foundation was supported by Grant 1 R01 CA-24629-01 from the National Cancer Institute, DHEW. The original line of Balb/c 3T3 cells used in this work was kindly provided by Dr. Takeo Kakunaga, National Institutes of Health. The authors acknowledge the skillful technical assistance of T. J. Goodwin.

Philip W. Le Quesne,* Adhyam V. Durga Vinayakam Subramanyam
Department of Chemistry and Department of Medicinal Chemistry and Pharmacology, Northeastern University Boston, Massachusetts 02115

Albert H. Soloway,* Ronald W. Hart
College of Pharmacy, The Ohio State University Columbus, Ohio 43210

Robert H. Purdy*
Southwest Foundation for Research and Education San Antonio, Texas 78284

Received May 11, 1979

Synthetic Modulators of the Complement System. 1. Synthesis and Biological Activity of 5,5′,5″-[1,3,6-Naphthalenetriyltris(sulfonylimino)]-tris[1,3-benzenedisulfonic acid] Hexasodium Salt

Sir:

Considerable progress has been made in defining the constituents of the complement system and elaborating their role in the lysis of cells and the inflammatory process.[1] In addition, complement has been implicated in the pathophysiology of a number of diseases, e.g., rheumatoid arthritis, lupus erythematosus, glomerulonephritis and periodontitis.[2]

A possible approach to the treatment of complement-dependent diseases involves the use of a synthetic chemical modulator (activator or inhibitor) to assist the body's

(11) Cornforth, J. W.; Green, D. T. *J. Chem. Soc. C* **1970**, 846.
(12) Cf. Hanson, J. R.; Wilkins, H. J. *J. Chem. Soc, Perkin Trans. 1* **1974**, 1388.
(13) Compound 13 had mp 143–144 °C; $[\alpha]_D$ (EtOH) −55°; IR (film) ν_{max} 3050, 1730, 1675, 1250, 1045, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.87 (3 H, s, C-18 Me), 2.03 (acetoxy Me), 3.3 (1 H, d, J = 2 Hz, 4-H), 4.67 (1 H, br t, 17-H), 5.93 (1 H, d, J = 10 and 2 Hz, 2-H), 6.70 (1 H dd, J = 10 and 2 Hz, 1-H).
(14) Compound 14 had mp 180–182 °C; $[\alpha]_D$ (EtOH) +149.6°; IR (film) ν_{max} 3020, 1725, 1670, 1620, 1250, 1045, 1025 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.90 (3 H, s, C-18 Me), 2.03 (3 H, acetoxy Me), 3.37 (1 H, dd, J = 4 and 2 Hz, 1-H), 3.70 (1 H, m, 2-H), 4.63 (1 H, br t, 17-H), 5.73 (1 H, br s, 4-H).
(15) Compare Mhaskar, D. N.; D'Ambrosio, S. M.; Modak, S. P.; Hart, R. W. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1979**, *38*, 484.
(16) Kakunaga, T. *Int. J. Cancer* **1973**, *12*, 463.

(1) (a) S. Ruddy, in "Mediators of Inflammation", G. Weissmann, Ed., Plenum Press, New York, 1974, Chapter 4; (b) P. A. Ward, in "Mechanisms of Immunopathology", S. Cohen, P. A. Ward, and R. T. McClusky, Eds., Wiley, New York, 1979, Chapter 1; (c) H. J. Müller-Eberhard, *Hosp. Practice*, 12, 33 (1977); (d) M. M. Mayer, *Harvey Lect.*, **72**, 139 (1977).
(2) (a) M. M. Frank and J. P. Atkinson, in "Disease-a-Month", Year Book Medical Publishers, Chicago, Ill., Jan 1975; (b) M. M. Frank, J. A. Gelfand, and J. P. Atkinson, *Ann. Intern. Med.*, 84, 580 (1976); (c) I. Gigli, in "Comprehensive Immunology", Vol. 2, N. K. Day and R. A. Good, Eds., Plenum Medical Book Co., New York, 1977, Chapter 15; (d) C. Rothback, R. L. Green, M. I. Levine, and P. Fireman, *Am. J. Med.*, 66, 681 (1979); R. J. Nisengard, *J. Periodontol.*, 48, 505 (1977); (e) N. J. Zvaifler, *Adv. Immunol.*, 16, 265 (1973); (f) A. Nicholson, D. T. Fearon, and K. F. Austen, *Handb. Exp. Pharmakol.*, 50(1), 425-463 (1978).

0022-2623/80/1823-0240$01.00/0

Table I. Complement Inhibition Assay Results

compd	in vitro assays: ED_{50} of compd, μg/mL: C-1[a] inhibitor	C-late[b] inhibitor	C-alt[c] inhibitor	cap 50[d] inhibitor, μg/mL (95% CL)	in vivo assay: guinea pig ip,[e] % inhibn (95% CL)
chlorazol FPB (I)	21.0	6	167	113 (97-135)	>95[f]
suramin (III)	21.0	50	167	603 (455-1019)	44 (34-53)
IIb	0.7	12	21	21 (16-79)	86 (72-93)

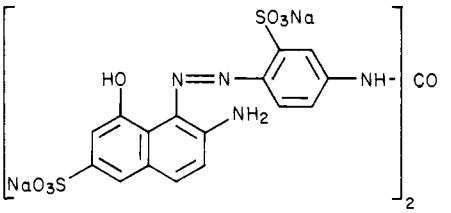
[a] C-1 assay: Measures the ability of activated fluid-phase human C1 to destroy human C2 in the presence of C4 and appropriate serial twofold dilutions of the test compound. Inhibition of C1 results in sparing of C2. [b] C-late assay: Measures the ability of the late components of human complement (C3-C9) to lyse EAC142 in the presence of appropriate serial twofold dilutions of the test compound. An active inhibitor protects EAC142 from lysis. [c] C-alt assay: Mercaptan-treated human erythrocytes [G. Sirchia, S. Ferrone, and F. Mercuriali, *Blood*, **25**, 502 (1965)] are lysed in autologous serum via the alternative pathway activated by cobra venom factor (*N. naja*) in the presence of appropriate serial twofold dilutions of the test compound. Inhibition of the alternative pathway results in failure of passive lysis. [d] cap 50 assay: The test compound is added to guinea pig serum in vitro after which complement activity is determined by the undiluted serum capillary tube assay method [J. A. Brockman and N. Bauman, *J. Immunol. Methods*, **27**, 353 (1979)]. The concentration of compound producing 50% inhibition is reported. [e] guinea pig ip assay: Guinea pigs weighing approximately 300 g are dosed ip with 200 mg/kg of test compound dissolved in saline and adjusted to pH 7-8. After 2 h, the guinea pigs are bled and the complement level is determined in undiluted serum by the above-cited capillary tube assay. [f] No complement detectable by this assay.

regulatory mechanism(s) in controlling the complement system. Indeed, many compounds of diverse structures have been reported that inhibit[3] and, in some cases, activate[4] this system.

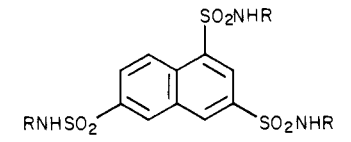
An in vitro screening program in our laboratories uncovered and confirmed a previously reported azo dye, Chlorazol Fast Pink B[5] (I), as a promising in vitro and in vivo inhibitor. We wish to record here that an extensive investigation of the modification of this structure has led to novel groups of polyanionic compounds that are strikingly active and do not contain the objectionable azo chromophore of I. These include the type of compound represented by the title polysulfonic acid, compound IIb.

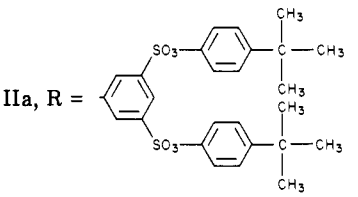
The synthesis of IIb was accomplished by standard chemical reactions employing an appropriate protective group for the acid functions. Thus, 5-nitrobenzenedisulfonic acid, disodium salt, was converted into its acid chloride in refluxing thionyl chloride–dimethylformamide[6] and, in turn, derivatized as the bis(*p*-*tert*-butylphenyl) ester. Reduction (10% Pd/C) gave the amine, which was condensed with 1,3,6-naphthalenetrisulfonyl chloride in pyridine to provide the hexaester, IIa. Facile removal of the protective groups was effected with sodium ethoxide in dimethyl sulfoxide to generate the desired IIb.

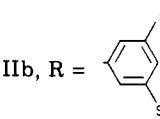
Biological. Table I illustrates the in vitro and in vivo complement-inhibitor properties of IIb compared to two

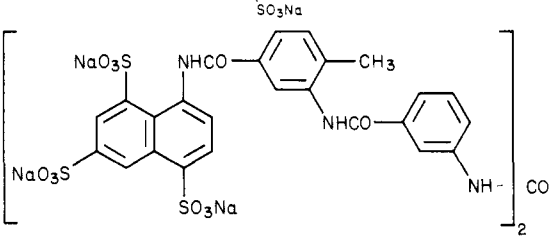


I









III

previously reported inhibitors, I and suramin sodium[7] (III). Thus, IIb is approximately 30 times more active than either I or III in the C-1 inhibitor assay and 5 to 30 times more active in suppressing hemolytic activity in undiluted serum. Compound IIb has been shown to potentiate the interaction of C-1 and human serum C-1 inhibitor.[8] The data of Table I show that it also exerts a lesser inhibitory

(3) Inter alios: (a) B. J. Johnson, *J. Pharm. Sci.*, **66**, 1367 (1977); (b) I. G. Otterness, A. J. Torchia, and H. D. Doshan, *Biochem. Pharmacol.*, **27**, 1873 (1978); (c) A. Kossorotow, W. Opitz, E. Etschenberg, and U. Hadding, *Biochem. J.*, **167**, 377 (1977); (d) J. M. Andrews, F. S. Rosen, S. J. Silverberg, M. Cory, E. E. Schneeberger, and D. H. Bing, *J. Immunol.*, **118**, 466 (1977); (e) Y. Ohsugi, T. Matsuno, and Y. Takagaki, *Chem. Pharm. Bull.*, **25**, 1202 (1977); (f) F. C. Berthoux, A.-M. Freyria, P.-Y. Zech, and J. Traeger, *Pathol. Biol.*, **25**, 33 (1977); (g) D. L. Brown, "Rheumatoid Arthritis", J. L. Gordon and B. L. Hazleman, Eds., Elsevier/North-Holland Biomedical Press, Amsterdam, 1977.

(4) Inter alios: (a) R. Burger, D. Bitter-Suermann, M. Loos, and U. Hadding, *Immunology*, **33**, 827 (1977); (b) C. M. Arroyave, M. Schatz, and R. A. Simon, *J. Allergy Clin. Immunol.*, **63**, 276 (1979).

(5) (a) H. P. Lambert and J. Richley, *Br. J. Exp. Pathol.*, **33**, 327 (1952); (b) N. Bauman and J. A. Brockman, *J. Immunol.*, **120**, 1764 (1978).

(6) H. H. Bosshard, R. Mory, M. Schmid, and H. Zollinger, *Helv. Chim. Acta*, **42**, 1653 (1959).

(7) J. S. C. Fong and R. A. Good, *Clin. Exp. Immunol.*, **10**, 127 (1972).

(8) N. Bauman and J. A. Brockman, *J. Immunol.*, in press.

action at other loci. In vivo, where pharmacology is superimposed upon intrinsic activity, IIb is substantially more effective than III and does not have the objectionable tissue-staining properties of I.

Acknowledgment. We thank the following for technical assistance: D. Colucci, W. Elliott, R. Pagano, B. Pease, and W. Pierce.

R. B. Conrow,* N. Bauman, J. A. Brockman S. Bernstein

Metabolic Disease Research Section Medical Research Division, American Cyanamid Company Lederle Laboratories, Pearl River, New York 10965

Received September 19, 1979

Articles

Synthesis and Enzymatic and Inotropic Activity of Some New 8-Substituted and 6,8-Disubstituted Derivatives of Adenosine Cyclic 3′,5′-Monophosphate

Jon P. Miller,

Department of Bioorganic Chemistry, Life Sciences Division, SRI International, Menlo Park, California 94025

Kay H. Boswell,

ICN Pharmaceuticals Inc., Irvine, California 92705

Rich B. Meyer, Jr.,

Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143

Leon F. Christensen, and Roland K. Robins*

Department of Chemistry, Cancer Research Center, Brigham Young University, Provo, Utah 84602.
Received September 14, 1979

The synthesis of certain new 8-(arythio)- and 8-(alkylthio)-cAMP derivatives and N^6-alkyl- and N^6-dialkyl-8-(arylthio) and -8-(alkylthio) derivatives of cAMP is reported. On the basis of activation of protein kinase, several N^6-alkyl-8-(benzylthio)-cAMP derivatives were selected for evaluation as inotropic agents using cat papillary muscle in vitro. Activity in these studies resulted in the selection of several analogues for in vivo studies in the anesthetized dogs. The best inotropic agent selected on the basis of in vivo studies was N^6-butyl-8-(benzylthio)-cAMP (**26**), which exhibited an increase in blood-flow rate of 85% with no increase in heart rate. A large-scale synthesis of **26** from cAMP is reported via N^1-alkylation, followed by a Dimroth rearrangement, reduction, bromination, and nucleophilic displacement via benzyl mercaptan. The N^6-alkyl-8-substituted-cAMP derivatives represent a new class of potent inotropic agents. The direct mechanism of action of **26** suggests the possible utility of this cyclic nucleotide to treat clinical myocardial infarction by rapid intravenous infusion.

The concept of designing nucleoside cyclic 3′,5′-monophosphates as potential medicinal agents was outlined in our first paper[1] in 1971. Progress of our studies in this area has recently been reviewed.[2,3] Amer and McKinney[4,5] have pointed out in some detail the potential of cyclic nucleotides as mediators of drug action in a number of areas of medicinal research. The possible sites in the system of cyclic nucleotide biochemistry which may serve as targets for drug design have recently been reviewed.[2,3,6] Our own approach to this field has been one of designing cyclic nucleotides which would either mimic or antagonize the action of cAMP or cGMP and which would be resistant to enzymatic degradation by phosphodiesterase[2,3] (PDE).

Ischemic heart disease is the most common serious health problem of our contemporary society.[7] In the United States alone, more than 675 000 patients die each year from ischemic heart disease and its complications. Approximately 1 300 000 patients each year have a myocardial infarction, and countless more suffer from congestive heart failure secondary to ischemic myocardial damage.[7] Thus, ischemic heart disease and its complications are by far the most common cause of death in the developed world.[7] The rapid decline in heart muscle contractility induced by ischemia is one of the major problems of clinical cardiovascular pharmacology. In 1962, Sutherland and co-workers[8] first suggested that cAMP was involved in the inotropic function of the heart when they showed that the in vivo potency of several catecholamines could be correlated with their ability to stimulate adenyl cyclase in membrane preparations of dog heart. Robison

(1) K. Muneyama, R. J. Bauer, D. A. Shuman, R. K. Robins, and L. N. Simon, *Biochemistry*, **10**, 2390 (1971).
(2) (a) J. P. Miller and R. K. Robins, *Annu. Rep. Med. Chem.*, 11, 291 (1976). (b) J. P. Miller, in "Cyclic 3′,5′-Nucleotides: Mechanisms of Action", H. Cramer and J. Schultz, Eds., Wiley, London, 1977, p 77. (c) R. B. Meyer, Jr., in "Burger's Medicinal Chemistry", 4th ed, Part II, M. E. Wolff, Ed., Wiley, New York, 1979, Chapter 34, 1201.
(3) R. K. Robins, *Med. Chem., Proc. Int. Symp., Invited Lect., 6th*, 1979, pp 127-136.
(4) M. S. Amer and G. R. McKinney, *Life Sci.*, **13**, 753 (1973).
(5) M. S. Amer and G. R. McKinney, *Annu. Rep. Med. Chem.*, **10**, 192 (1975).
(6) M. S. Amer, *Adv. Drug Res.*, **12**, 1 (1977).
(7) L. D. Hillis and E. Braunwald, *N. Engl. J. Med.*, **296**, 971 (1977).
(8) F. Murad, Y. M. Chi, T. W. Rall, and E. W. Sutherland, *J. Biol. Chem.*, **237**, 1223 (1962).

0022-2623/80/1823-0242$01.00/0