(17) E. F. J. Duynstee, J. L. J. P. Henneken, and M. R. A. H. Mevis, *Rec. Trav. Chim. Pays-Bas*, **84**, 1442 (1965).
(18) A. Ahmad, *Bull. Chem. Soc. Jpn.*, **47**, 2583 (1974).
(19) A. W. Coulter, J. B. Lombardini, J. R. Sufrin, and P. Talalay, *Mol. Pharmacol.*, **10**, 319 (1974).
(20) H. T. Nagasawa, J. G. Kohlhoff, P. S. Fraser, and A. A. Mikhail, *J. Med. Chem.*, **15**, 483 (1972).
(21) G. W. Smith and H. D. Williams, *J. Org. Chem.*, **26**, 2211 (1961).
(22) O. Noren, H. Sjöström, and L. Josefson, *Biochim. Biophys. Acta*, **327**, 446 (1973).
(23) S. Udenfriend, S. Stein, P. Böhlen, W. Dairman, W. Leimgruber, and W. Weigele, *Science*, **178**, 871 (1972).
(24) A. Meister, *ibid.*, **180**, 33 (1973).
(25) S. S. Tate and A. Meister, *J. Biol. Chem.*, **249**, 7593 (1974).
(26) L. Neelakantan and W. H. Hartung, *J. Org. Chem.*, **23**, 964 (1958).

ACKNOWLEDGMENTS

Supported by a program grant from the Veterans Administration.
The authors thank J. O. McMahon for the electron-impact spectra, Mrs. O. Hammerston for the IR spectra, and Dr. R. Vince for the P-388 leukemia cells. Dr. Harry B. Wood, Jr., Drug Research and Development Branch, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, kindly provided the *in vivo* antitumor screening data.

Anti-Inflammatory Activity of Amine Cyanoboranes, Amine Carboxyboranes, and Related Compounds

IRIS H. HALL *x, C. O. STARNES *, A. T. McPHAIL ‡, P. WISIAN-NEILSON ‡, M. K. DAS ‡, F. HARCHELROAD, Jr. ‡, and B. F. SPIELVOGEL ‡

Received April 10, 1979, from the **Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514,* and the ‡*Paul M. Gross Chemical Laboratory, Duke University, Durham, NC 27706.* Accepted for publication July 20, 1979.

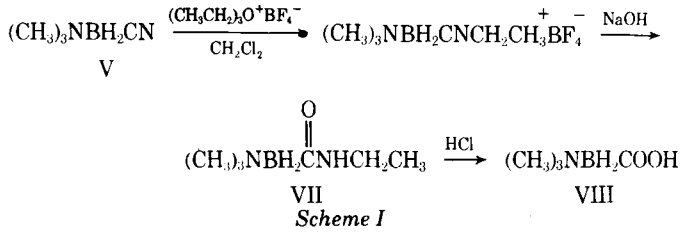
Abstract □ Amine cyanoboranes and amine carboxyboranes (boron analogs of α-amino acids) were shown to inhibit inflammation. The analogs effectively blocked general inflammation, induced arthritis, and the writhing reflex associated with inflammation pain, while the inflammation associated with pleurisy was marginally inhibited. The boron analogs were shown *in vitro* to inhibit the release of lysosomal enzymes from liver and polymorphonuclear neutrophils. Furthermore, prostaglandin synthesis was blocked by these agents at a low concentration, *i.e.*, 10^{-6} *M*. Liver oxidative phosphorylation processes also were uncoupled by these agents, but the migration of polymorphonuclear neutrophils was unaltered at 10^{-4} *M*. The elevation of cyclic adenosine monophosphate levels in polymorphonuclear neutrophils correlated positively with *in vivo* antiarthritic activity. Initial studies in rodents demonstrated that these boron analogs can be used at safe therapeutic doses.

Keyphrases □ Anti-inflammatory activity—evaluation of amine cyanoboranes, amine carboxyboranes, and related compounds □ Amine cyanoboranes and amine carboxyboranes—evaluation for anti-inflammatory activity

The antineoplastic activity of some α-amino boron analogs was reported previously (1). While studying their metabolic effects on tumor cell metabolism, it was noted that these agents interfered with oxidative phosphorylation processes of mitochondria, inhibited lysosomal enzymatic hydrolytic activities, and elevated cyclic adenosine monophosphate levels. Since commercially available anti-inflammatory agents, *e.g.*, phenylbutazone, salicylates, and indomethacin, have similar effects on cellular metabolism, testing of the boron analogs for anti-inflammatory activity in rodents was undertaken and the data are reported here.

EXPERIMENTAL

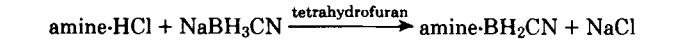
Chemistry—Consideration of the isoelectronic formalism between carbon and boron results in the prediction of boron analogs of dipolar α-amino acids, *e.g.*, glycine ammonia carboxyborane, alanine ammonia carboxymethylborane, and betaine triethylamine carboxyborane. Interest in these boron analogs lies mainly in their potential biological activity when compared with the enormous biological activity of the α-amino acids. A highly significant step toward demonstrating the existence of this class of compounds was the synthesis of trimethylamine carboxyborane, the protonated boron analog of betaine (2) (Scheme I).



Scheme I

Trimethylamine carboxyborane, a white, crystalline solid whose X-ray crystal structure was determined, is stable in air and water.

Amine Cyanoboranes—One approach to the synthesis of boron analogs of the α-amino acids involves the conversion of an amine cyanoborane to a boroamino acid according to the procedure outlined in Scheme I. To provide adequate quantities of the precursor amine cyanoboranes, a general, convenient, high-yield synthesis (3, 4) of this class of compounds was developed (Scheme II).



Scheme II

All previous syntheses (5–9) of amine cyanoboranes were limited as to the yield and reaction scale. Several synthetic procedures were developed (5, 6) following initial reports from these laboratories (10, 11) on the preparation of cyanoborane oligomers, *i.e.*, $(BH_2CN)_x$, by the addition of dry hydrogen chloride to a solution containing cyanohydroborate in ether. Thus, addition of amines to solutions of cyanoborane [which also can be prepared (8) by the reaction of cyanohydroborate and halogens] gives the amine cyanoborane. Typical yields have been ~25%, whereas yields up to 90% have been obtained with the amine hydrochloride procedure.

Following Scheme II, amine cyanoboranes were prepared where the amine was trimethylamine (V), dimethylamine (VI), pyridine (XV), 3-dimethylaminopropionitrile (XIX), or *N*-methylmorpholine (XIV). Bis(cyanoboranes) (XI and XX) were prepared (4) from ethylenediamine and tetramethylethylenediamine. However, attempts to prepare the parent ammonia cyanoborane (XVII) by this procedure were not successful. The only other report of the preparation of XVII (7) involved the reaction of trimethylamine iodoborane (IX) with sodium cyanide in liquid ammonia. Attempts to repeat this reaction in these laboratories resulted

0022-3549/80/0900-1025$01.00/0

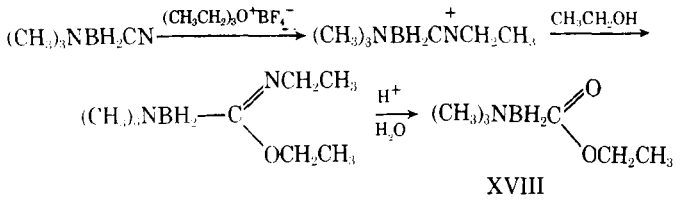
instead in the isolation of the novel complex hexakis(ammonia cyanoborane)sodium iodide (X) (12). The complex, which was characterized by X-ray crystallography, consists of six ammonia cyanoborane (XVII) molecules coordinated octahedrally around the sodium cation. However, pure ammonia cyanoborane could be obtained by dissolution of the complex in water and extraction of the ammonia cyanoborane into ether (13). The compound also was prepared (13) by base displacement (Scheme III).

$$C_6H_5NH_2{\cdot}BH_2CN \xrightarrow{H_3N} \underset{\text{XVII}}{H_3NBH_2CN} + C_6H_5NH_2$$

Scheme III

In both cases, ammonia cyanoborane as prepared in these laboratories differs from that reported previously (7), which was characterized only partially. Ammonia cyanoborane possesses considerable hydrolytic stability, suffering only an 8% loss of hydrogen in concentrated hydrochloric acid after 70 hr at room temperature. In general, the amine cyanoboranes are quite stable to air and moisture.

Amine Carboxyboranes—By using the general synthetic route illustrated in Scheme I, amine carboxyborane compounds where the amine was pyridine (XVI) or *N*-methylmorpholine were prepared (4) from the corresponding amine cyanoborane. Tetramethylethylenediamine bis(carboxyborane) (XII) and its intermediate (XIII) were synthesized (4). Trimethylamine carboxyborane (VIII) derivatives can be prepared readily by the preparation of the ethyl ester. The ethyl ester, a white solid (mp 45–47°), can be prepared in a 34% yield according to Scheme IV.



Scheme IV

The ethyl ester was obtained by allowing a solution of 95% ethanol (200 ml), concentrated aqueous hydrochloric acid (8 ml), and the *N*-ethylnitrilium salt [prepared by refluxing for 24 hr a solution of trimethylamine cyanoborane (0.2 mole) and 400 ml of 1 *N* triethyloxonium tetrafluoroborate in methylene chloride (2)] to reflux for 48 hr. After neutralization with a saturated sodium bicarbonate solution and removal of ethanol under reduced pressure, the product was extracted from the aqueous solution with methylene chloride. Drying the organic portion over magnesium sulfate and solvent removal produced a yellow liquid. Purification by recrystallization from ethanol or ether or by sublimation afforded the ester as a white crystalline solid (9.9 g). Satisfactory carbon, hydrogen, nitrogen, and boron analyses were obtained[1]. The PMR signals (deuterochloroform) were those expected for the trimethylamine (δ 2.78 singlet) and ethyl (δ 1.2 triplet and δ 4.10 quartet) functions; strong B–H (2380 cm^{-1}) and C=O (1660 cm^{-1}) stretches were present in the IR spectrum.

The structures of the boron analogs and references for their syntheses are given in Table I.

Pharmacology—*Toxicity Studies*—Acute LD_{50} toxicity studies were carried out in CF_1 male mice (~30 g) using the method of Litchfield and Wilcoxon (14).

Anti-Inflammatory Screen—CF_1 male mice (~30 g) were administered the test drugs at 10 mg/kg ip in 0.05% polysorbate 80–water 3 hr and then 30 min prior to injection of 0.05 ml of 2% carrageenan in 0.9% saline into the plantar surface of the right hindfoot. Saline injected into the left hindfoot served as a baseline. After 3 hr, both feet were excised at the tibiotarsal (ankle) joint according to the modified (15) method of Winter, resulting in a net weight increase of 87 mg in the feet of the control animals.

Antipyretic Screen—Sprague–Dawley rats (~200 g) were administered 2 ml of a 44% solution of baker's yeast subcutaneously (15) 18 hr prior to the injection of drugs at 2.5 or 5 mg/kg ip, resulting in an elevation of 3.46° F. Alternatively, rats were administered 0.25 mg of killed and dried *Mycobacterium butyricum*[2] (16) subcutaneously prior to drug administration. Rectal temperatures were taken immediately before and 2, 4, and 6 hr after drug administration.

Writhing Reflex—CF_1 male mice were administered the test drugs at 20 mg/kg ip 20 min (17) prior to the administration of 0.5 ml of 0.6% acetic acid (18). After 5 min, the number of stretches, characterized by repeated contractures of the abdominal musculature accompanied by hindlimb extension, was counted for the next 10 min. Control mice had 78 stretch reflexes/10 min.

Chronic Adjuvant Arthritic Screen—Male Sprague–Dawley rats (~160 g) were injected at the base of the tail with 0.2 ml of a solution of killed and dried *M. butyricum* and digitonin in mineral oil (19). Test drugs were administered intraperitoneally on Days 3–20 at 2.5 mg/kg/day. Animals were sacrificed on Day 21, and the feet were excised and weighed. Control animals had a net weight gain of 0.830 g.

Antipleurisy Screen—Sprague–Dawley rats were administered the test drugs at 2.5 mg/kg ip 1 hr before injection and 3 hr postinjection of 0.05 ml of a solution of 0.316% Evan's blue and carrageenan into the pleural cavity (20). After 6 hr, the rats were sacrificed and the fluid was collected from the pleural cavity. Control rats produced 2.5 ml of fluid.

Table I—Structures of Boron Analogs

Compound	Formula	Synthetic Reference
I	$(CH_3)_3CNH_2{\cdot}BH_3$[a]	—
II	$O(CH_2CH_2)_2NH{\cdot}BH_3$[a]	—
III	$(CH_3)_3N{\cdot}BH_3$[b]	—
IV	$(CH_3)_2NH{\cdot}BH_3$[b]	—
V	$(CH_3)_3N{\cdot}BH_2CN$	1, 3
VI	$(CH_3)_2NH{\cdot}BH_2CN$	1, 3
VII	$(CH_3)_3N{\cdot}BH_2CONHCH_2CH_3$	1, 2
VIII	$(CH_3)_3N{\cdot}BH_2COOH$	1, 2
IX	$(CH_3)_3N{\cdot}BH_2I$	1, 6
X	$[Na[H_3N{\cdot}BH_2(CH)]_6]I$	1, 12
XI	$[CH_2N(CH_3)_2{\cdot}BH_2CN]_2$	4
XII	$[CH_2N(CH_3)_2{\cdot}BH_2COOH]_2$	4
XIII	$[CH_2N(CH_3)_2{\cdot}BH_2CONHCH_2CH_3]_2$	4
XIV	$O(CH_2CH_2)_2N(CH_3){\cdot}BH_2CN$	3–5
XV	$C_5H_5N{\cdot}BH_2CN$	3–5
XVI	$C_5H_5N{\cdot}BH_2COOH$	4
XVII	$H_3N{\cdot}BH_2CN$	13
XVIII	$(CH_3)_3N{\cdot}BH_2COOCH_2CH_3$	Text
XIX	$NCCH_2CH_2N(CH_3)_2{\cdot}BH_2CN$	4
XX	$(CH_2NH_2{\cdot}BH_2CN)_2$	4

[a] Alfa Products. [b] Aldrich Chemicals.

Table II—*In Vivo* Anti-Inflammatory Activity of Boron Analogs

Compound	LD_{50}, mg/kg	Percent of Control: Anti-Inflammatory Screen, 10 mg/kg × 2	Percent of Control: Writhing Reflex, 20 mg/kg	Percent of Control: Antiarthritic Screen, 2.5 mg/kg/day
I	16	41	—	—
II	475	55	15	33
III	740	65	47	12
IV	200	66	37	61
V	70	42	18	4
VI	39	49	24	25
VII	320	63	29	64
VIII	1800	79	29	53
IX	250	66	37	22
X	100	57	12	0
XI	200	95	76	77
XII	>1000	58	54	19
XIII	>1000	65	55	24
XIV	23	91[a]	78[a]	16
XV	25	83[a]	80	45
XVI	>200	74[a]	53	44
XVII	30	51	50	32
XVIII	>500	87	106	26
XIX	140	74	89	13
XX	>150	87	95	100
Indomethacin[b]	28	22	43	27
Control	—	100	100	100

[a] One-third dose. [b] Tested at 10 mg/kg.

[1] *Anal.*—Calc.: C, 49.70; H, 11.12; B, 7.46; N, 9.66. Found: C, 49.66; H, 11.04; B, 7.56; N, 9.56.

[2] Difco.

Table III—Effects of Boron Analogs on *In Vitro* Lysosomal Enzymatic Activities and Cyclic Adenosine Monophosphate Levels at 5 μMoles

Compound	Percent of Control				
	Liver		Polymorphonuclear Neutrophils		
	Percent of Free Acid Phosphatase Activity (pH 5)	Percent of Free Cathepsin Activity (pH 5)	Percent of Free Acid Phosphatase Activity (pH 5)	Percent of Free Cathepsin Activity (pH 5.0)	Cyclic Adenosine Monophosphate Level
I	77 ± 4	58 ± 5	100 ± 9	100 ± 4	58
II	75 ± 8	107 ± 6	79 ± 5	93 ± 7	107
III	72 ± 6	17 ± 4	57 ± 4	18 ± 3	145
IV	82 ± 3	67 ± 3	82 ± 6	65 ± 5	80
V	69 ± 4	0 ± 2	35 ± 5	4 ± 2	245
VI	73 ± 4	32 ± 5	89 ± 4	22 ± 1	98
VII	78 ± 7	—	100 ± 2	22 ± 5	98
VIII	82 ± 5	56 ± 6	50 ± 3	14 ± 4	99
IX	71 ± 6	14 ± 3	71 ± 5	40 ± 4	133
X	65 ± 3	3 ± 1	34 ± 6	0 ± 3	361
XI	80 ± 6	50 ± 7	84 ± 5	16 ± 5	94
XII	69 ± 5	23 ± 6	84 ± 4	52 ± 4	155
XIII	74 ± 4	—	100 ± 7	70 ± 6	92
XIV	68 ± 7	13 ± 5	50 ± 3	37 ± 5	165
XV	79 ± 3	57 ± 4	57 ± 4	49 ± 7	110
XVI	77 ± 8	58 ± 4	100 ± 5	31 ± 4	100
XVII	75 ± 5	48 ± 6	100 ± 2	26 ± 4	93
XVIII	—	—	—	16 ± 3	—
XIX	66 ± 4	15 ± 3	100 ± 9	17 ± 5	199
XX	77 ± 8	80 ± 7	100 ± 10	57 ± 2	28
Indomethacin	86 ± 4	67 ± 6	100 ± 2	100 ± 1	80
Control	100 ± 7[a]	100 ± 6[b]	100 ± 8[c]	100 ± 6[d]	100[e]

[a] 0.753 mg of inorganic phosphate released/hr/g of wet tissue. [b] 2.69 mg of protein released/hr/g of wet tissue. [c] 0.26 μg of inorganic phosphate released/hr/10^7 cells. [d] 0.225 mg of protein released/hr/10^7 cells. [e] 48 pmoles of cyclic adenosine monophosphate/10^7 cells.

Polymorphonuclear neutrophil migration studies were carried out according to Nelson *et al.* (21). The analogs were tested *in vitro* at 10^{-4} *M*.

Ulcerogenic tests were carried out in Sprague–Dawley rats (~160 g); they were administered II, V, X, XIV, and XIX at 2.5 mg/kg for 3 weeks. After the rats were fasted for 18 hr, the last dose on Day 21 was administered. After 4 hr, the rats were sacrificed and the gastric and duodenum mucosa were examined for bleeding and ulcers (22). In an analogous experiment, animals were bled on Day 21 by the tail vein; the red and white blood cells were counted in a hemocytometer and expressed as the number of cells × 10^6 per cubic centimeter. Hematocrits also were obtained (23).

Biochemistry—*Lysosomal Enzymatic Assays*—Sprague–Dawley rats (~160 g) were sacrificed, the liver was excised, and a 10% homogenate (four times) in 0.25 *M* sucrose and 0.001 *M* ethylenediaminetetraacetic acid (pH 7.2) was prepared.

Polymorphonuclear neutrophils were collected from the peritoneal cavity 4 hr after injection of 35 ml of 0.5% oyster glycogen in isotonic saline (24). The polymorphonuclear neutrophils were centrifuged at 800×*g* for 20 min, washed, and resuspended in minimum essential medium with 10% fetal calf serum (pH 7.4).

Acid phosphatase activity (25) was determined by incubating 0.1 *M* β-glycerol phosphate in 0.1 *M* acetate (pH 5.0) with 5 μmoles of the test drugs in 1% carboxymethylcellulose sodium for 20 min with the liver homogenate and for 60 min with the resuspended polymorphonuclear neutrophils. Lysosome enzymes were released by 0.02% alkylphenoxy polyethoxyethanol 100[3] to obtain the total enzymatic activity. The reaction was stopped with 10% trichloroacetic acid, and the solution was centrifuged. Aliquots of the supernate were assayed for inorganic phosphate content by the method of Chen *et al.* (26). The percentage of free acid phosphatase was calculated.

Cathepsin activity was determined in an analogous manner, except that the substrate was 2% azocasein (27) and 0.1 *M* acetate buffer (pH 5.0). The supernate was assayed for acid-soluble peptide fragments at 366 nm. The percentage of free cathepsin activity was calculated.

Cyclic Adenosine 3′,5′-Monophosphate Levels—Isolated polymorphonuclear neutrophils were incubated with the test drugs at 5 μmoles for 1 hr at 37° in minimum essential medium at pH 7.4. The reaction was stopped with 6% trichloroacetic acid. The cyclic-3′,5′-adenosine monophosphate levels were determined by the radioimmunoassay of Gilman (28) using [^{3}H(G)]cyclic adenosine 3′,5′-monophosphate (39.8 Ci/mmole). Results were calculated in picomoles per 10^7 polymorphonuclear neutrophils.

Prostaglandin Synthetase Activity—The incubation medium of Tomlinson *et al.* (29) was used to determine [^{3}H]prostaglandin formation (prostaglandins E, F_α, and D) from [^{3}H(N)]arachidonic acid (86.2 Ci/mmole) and 10 mg of purified commercial prostaglandin synthetase from beef seminal vesicles[4]. After 1 hr at 37°, the reaction was stopped with 1 *N* HCl and the solution was extracted with ether and evaporated. The residue was taken up in ethyl acetate and spotted on silica gel TLC plates, which were eluted with chloroform–methanol–water–acetic acid (90:8:1:0.8) (30). The plates were dried and developed in iodine vapor; with the use of prostaglandin standards, the appropriate areas were scraped and counted for tritium content. Indomethacin was used as a standard at 10^{-4} *M*. The test drugs were used at 10^{-6} *M*.

Oxidative Phosphorylation Studies—The basal respiration and adenosine diphosphate-stimulated respiration on 10% liver homogenates were measured using succinate or α-ketoglutarate as the substrate (31). The reaction vessel contained sucrose (55 μmoles), potassium chloride (22 μmoles), dibasic potassium phosphate (22 μmoles), test compounds at 5 μmoles in 1% carboxymethylcellulose sodium and sodium succinate (90 μmoles) or α-ketoglutarate (60 μmoles) in a total volume of 1.8 ml. After the basal metabolic (State 4) level was obtained, 0.257 μmole of adenosine diphosphate was added to obtain the adenosine diphosphate-stimulated rate (State 3). The respiration rate was calculated as microliters of oxygen consumed per hour per milligram (wet weight) of liver.

RESULTS

The boron analogs afforded significant anti-inflammatory activity in rodents. Administration of I, V, and VI resulted in at least 50% inhibition of the carrageenan-induced edema in mouse footpads (Table II), while II, X, XII, and XVII resulted in at least 40% inhibition. Administration of II, V, and X caused 80% inhibition of the writhing reflex, which is similar to inflammation pain. Compounds VI–VIII caused 70% inhibition of the reflex. In the induced arthritic screen in rats, 3 weeks of dosing of III, V, X, XIV, and XIX caused >80% inhibition, with X and V causing 100 and 96% inhibition, respectively. Compound V was administered at 1.25–10 mg/kg/day ip. Doses at 1.25 mg/kg/day caused 75% inhibition, doses of 2.5 mg/kg/day caused 96% inhibition, and doses of 5 or 10 mg/kg/day resulted in 100% inhibition. Compounds VI, IX, XIII, and XVIII

[3] Triton X-100.

[4] Miles Research Products.

Table IV—Effects of Boron Analogs on *In Vitro* Prostaglandin Synthesis and Oxidative Phosphorylation Processes

Compound	Percent of Control				
	Prostaglandin Synthesis, × 10^{-6} *M*	Oxidative Phosphorylation (5 μmoles)			
		Succinate		α-Ketoglutarate	
		State 4	State 3	State 4	State 3
I	100 ± 3	118 ± 11	63 ± 8	160 ± 35	63 ± 14
II	100 ± 5	115 ± 15	66 ± 10	97 ± 29	50 ± 11
III	61 ± 3	117 ± 11	56 ± 12	71 ± 8	28 ± 10
IV	100 ± 5	119 ± 9	82 ± 4	67 ± 18	53 ± 16
V	59 ± 6	133 ± 20	51 ± 20	85 ± 21	42 ± 9
VI	63 ± 4	126 ± 19	61 ± 5	128 ± 25	45 ± 12
VII	100 ± 7	114 ± 13	74 ± 9	113 ± 37	45 ± 18
VIII	93 ± 3	122 ± 14	63 ± 4	127 ± 22	70 ± 20
IX	67 ± 5	120 ± 12	63 ± 9	102 ± 16	70 ± 13
X	42 ± 5	110 ± 5	31 ± 10	101 ± 25	37 ± 10
XI	85 ± 4	116 ± 8	122 ± 13	168 ± 38	106 ± 21
XII	60 ± 6	80 ± 11	57 ± 16	122 ± 15	48 ± 30
XIII	100 ± 4	117 ± 8	66 ± 8	162 ± 43	50 ± 24
XIV	38 ± 3	62 ± 10	36 ± 5	140 ± 47	51 ± 16
XV	58 ± 3	124 ± 16	57 ± 16	112 ± 42	52 ± 15
XVI	76 ± 5	110 ± 13	75 ± 15	109 ± 30	63 ± 15
XVII	55 ± 6	112 ± 7	60 ± 11	149 ± 27	40 ± 9
XVIII	—	119 ± 18	61 ± 19	126 ± 51	54 ± 8
XIX	53 ± 7	43 ± 16	24 ± 7	105 ± 33	33 ± 8
XX	81 ± 5	13 ± 5	10 ± 5	50 ± 18	25 ± 8
Indomethacin (10^{-4} *M*)	64 ± 2	—	—	—	—
Control	100[a]	100 ± 6[b]	100 ± 4[c]	100 ± 8[d]	100 ± 9[e]

[a] 6564 dpm of prostaglandin E formed/hr/mg of enzyme. [b] 9.19 μl of oxygen consumed/hr/mg of wet tissue. [c] 13.66 μl of oxygen consumed/hr/mg of wet tissue. [d] 3.38 μl of oxygen consumed/hr/mg of wet tissue. [e] 4.93 μl of oxygen consumed/hr/mg of wet tissue.

afforded >70% inhibition of the induced arthritic state, and II and XVII caused 60% inhibition.

In the antipyretic test, V at 5 or 10 mg/kg caused no inhibition of elevated body temperature. In the antipleurisy screen, inhibition of 33, 35, 49, and 39% was demonstrated for III, V, X, and XIX, respectively. Boron analogs had no effect on the chemotactic migration of polymorphonuclear neutrophils. Dosing for 3 weeks with II, V, X, XIV, and XIX intraperitoneally resulted in no alteration of the red or white blood cell count per cubic millimeter and in no gastric or duodenum mucosa irritations or bleeding.

The percentage of free lysosomal enzymatic activities in the liver and polymorphonuclear neutrophils was inhibited by the presence of boron analogs (Table III). Free acid phosphatase activity in the liver was inhibited 28–35% by III, V, IX, X, XII, XIV, and XIX; in polymorphonuclear neutrophils, inhibition of 43, 65, 50, 66, and 50% was found for III, V, VIII, X, and XIV, respectively. Free proteolytic cathepsin activity of the liver was inhibited at least 75% by III, IX, XII, XIV, and XIX, with 100% inhibition by V and 97% inhibition by X. Inhibition of polymorphonuclear neutrophil cathepsin activity of at least 82% was exhibited by III, VIII, X, XI, XVIII, and XIX, with 96% inhibition by V and 100% inhibition by X.

Cyclic adenosine monophosphate levels in polymorphonuclear neutrophils were elevated after *in vitro* incubation with drugs (Table III). The increases were 45, 145, 33, 261, 55, 65, and 99% for III, V, IX, X, XII, XIV, and XIX, respectively.

At 10^{-6} *M in vitro*, III, V, VI, IX, X, XII, XIV, XV, XVII, and XIX blocked prostaglandin synthesis by >30% (Table IV). Compound X resulted in 58% inhibition, and XIV caused 62% inhibition. The presence of boron analogs at 5 μmoles caused uncoupling of the oxidative phosphorylation processes of liver mitochondria. Several agents (*e.g.*, XX) suppressed basal (State 4) as well as adenosine diphosphate-stimulated respiration (State 3) with both substrates. Uncoupling of the electron transport chain was seen with the substrate succinate, a flavin adenine dinucleotide-linked dehydrogenase, with I–X, XIII, and XV–XVIII. This uncoupling was observed as an increase in State 4 respiration and as a decrease in State 3 respiration. With α-ketoglutarate as the substrate, a nicotinamide diphosphate-linked dehydrogenase, uncoupling was seen with I, VI–VIII, and XII–XVIII. Both States 3 and 4 respiration were inhibited with succinate in the presence of XII, XIV, XIX, and XX and with α-ketoglutarate by III–V and XX. Compounds that demonstrated potent antiarthritic activity also demonstrated potent inhibitory effects on State 3 respiration, *e.g.*, III, V, X, XII, XIV, and XIX with either succinate or α-ketoglutarate. The exception to this observation was XX.

DISCUSSION

Inflammation is a process associated with the release of chemical vasoamines, *e.g.*, histamine, serotonin, slow-reacting substance, and bradykinin, the release of lysosomal hydrolytic enzymes by leukocytes, the synthesis and release of prostaglandins, and the modulation of cyclic nucleotide levels of lymphocytes and leukocytes (32–36). These agents, in turn, cause increased vascular permeability, chemotaxis of polymorphonuclear neutrophils and macrophages, erythema, dermatitis, hypersensitivity, complement fixation, allergy reactions, and edema, and they elicit pain together with increasing the local and body temperature.

The commercially available anti-inflammatory agents are known to interfere with some of these processes, thus retarding the development of inflammation. For example, indomethacin decreases the polymorphonuclear neutrophil motility, uncouples oxidative phosphorylation processes, inhibits prostaglandin synthetase and histidine decarboxylase activities, mucopolysaccharide biosynthesis, and platelet function, stabilizes lysosomal membranes, and thus inhibits hydrolytic enzymatic activity (32). A number of agents are known to inhibit cyclic adenosine monophosphate phosphodiesterase activity (37) and increase cyclic adenosine monophosphate, which supposedly stabilizes the lysosomal membranes, blocks immunoglobulin E-dependent antigen-induced vasoamine release, and inhibits hypersensitivity (38–43).

As seen from the current studies, compounds having potent antiarthritic effects, *e.g.*, III, V, X, XII, XIV, and XIX, also were significant inhibitors of lysosomal-free hydrolytic activity both from the liver and polymorphonuclear neutrophils, indicating membrane stabilization. The inhibition of cathepsin activity was particularly high by the boron analogs. Increased levels of activity of this enzyme have been linked with a number of inflammation states. The boron analogs at the same concentration as indomethacin were more effective in inhibiting polymorphonuclear neutrophil lysosomal rupture.

Prostaglandin synthetase activity also was suppressed by III, V, X, XII, XIV, and XX at 10^{-6} *M*. Indomethacin at 10^{-6} *M* resulted in only 24% inhibition in this system; at 10^{-4} *M*, indomethacin caused 36% inhibition. Analogs V, X, XIV, XV, XVII, and XIX were more potent than indomethacin in inhibiting prostaglandin synthesis by the isolated enzyme system. Oxidative phosphorylation processes were uncoupled at 5 μmoles for the boron compounds, which demonstrated potent *in vivo* antiarthritic activity in rats. The uncoupling and suppression of energy production needed for migration and phagocytosis of polymorphonuclear neutrophils and macrophages were seen with all of the boron analogs; however, the migration of polymorphonuclear neutrophils at 10^{-4} *M* demonstrated no inhibition by boron analogs. Thus, it was more difficult to demonstrate positive correlation of uncoupling of respiration with *in vivo* antiarthritic activity. Nevertheless, excluding XX, those compounds possessing potent antiarthritic activity all suppressed adenosine diphosphate-stimulated respiration (State 3) by 40% or better.

The effects of boron analogs on respiration may be secondary to other metabolic effects. A more positive correlation with *in vivo* antiarthritic

activity is seen with the ability of the drug to elevate intracellular levels of cyclic adenosine monophosphate, which could account for the ability of these agents to block lysosomal enzyme release and prostaglandin synthesis and release. Elevated levels of cyclic adenosine monophosphate have been correlated with the ability to block the release of lysosomal enzymes (25, 41). The exact mechanism by which boron analogs cause elevations in cyclic adenosine monophosphate levels needs further study. Toxicity and side effects did not appear to be problems with boron analogs at the required therapeutic doses.

REFERENCES

(1) I. H. Hall, C. O. Starnes, B. F. Spielvogel, P. Wisian-Neilson, M. K. Das, and L. Wojnowich, *J. Pharm. Sci.*, **68,** 685 (1979).

(2) B. F. Spielvogel, L. Wojnowich, M. K. Das, A. T. McPhail, and K. D. Hargrave, *J. Am. Chem. Soc.*, **98,** 5702 (1976).

(3) P. Wisian-Neilson, M. K. Das, and B. F. Spielvogel, *Inorg. Chem.*, **17,** 2327 (1978).

(4) B. F. Spielvogel, P. Wisian-Neilson, and F. Harchelroad, Jr., *J. Inorg. Nucl. Chem.*, in press.

(5) S. S. Uppal and H. C. Kelly, *Chem. Commun.*, **1970,** 1619.

(6) C. Weidig, S. S. Uppal, and H. C. Kelly, *Inorg. Chem.*, **13,** 1763 (1974).

(7) P. J. Bratt, M. P. Brown, and K. R. Seddon, *J. Chem. Soc. Dalton Trans.*, **1974,** 2161.

(8) D. R. Martin, M. A. Chiusano, M. L. Denniston, D. J. Dye, E. D. Martin, and B. T. Pennington, *J. Inorg. Nucl. Chem.*, **40,** 9 (1978).

(9) O. T. Beachley and B. Washburn, *Inorg. Chem.*, **14,** 120 (1975).

(10) B. F. Spielvogel, J. M. Purser, and C. G. Moreland, *Chem. Eng. News,* **47**(47), 38 (Nov. 10, 1969).

(11) B. F. Spielvogel, R. F. Bratton, and C. G. Moreland, *J. Am. Chem. Soc.*, **94,** 8567 (1972).

(12) K. D. Hargrave, A. T. McPhail, B. F. Spielvogel, and P. Wisian-Neilson, *J. Chem. Soc. Dalton Trans.*, **1977,** 2150.

(13) A. T. McPhail, K. D. Onan, B. F. Spielvogel, and P. Wisian-Neilson, *J. Chem. Res.* (S), **205** (M), 2601 (1978).

(14) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96,** 99 (1949).

(15) A. P. Roszkowski, W. H. Rooks, A. Tomolonis, and L. M. Miller, *ibid.*, **179,** 114 (1971).

(16) L. O. Randall and H. Baruth, *Arch. Int. Pharmacodyn. Ther.*, **220,** 94 (1976).

(17) L. C. Hendershat and J. Forsaith, *J. Pharmacol. Exp. Ther.*, **125,** 237 (1959).

(18) R. Vingar, J. F. Traux, and J. L. Selph, *Eur. J. Pharmacol.*, **37,** 23 (1976).

(19) B. H. Waksman, C. M. Pearson, and J. T. Sharp, *J. Immunol.*, **85,** 403 (1960).

(20) L. F. Sancilio, *Proc. Soc. Exp. Biol. Med.*, **127,** 597 (1968).

(21) B. D. Nelson, P. G. Quie, and R. L. Simmons, *J. Immunol.*, **115,** 1650 (1975).

(22) D. A. Brodie and B. J. Chase, *J. Gastroenterol.*, **53,** 604 (1967).

(23) S. A. Lang, B. D. Johnson, E. Cohen, A. E. Sloboda, and E. Greenblatt, *J. Med. Chem.*, **19,** 1404 (1976).

(24) R. P. Orange, M. D. Valentine, and K. F. Austen, *J. Exp. Med.*, **127,** 767 (1968).

(25) R. Gianetto and C. de Duve, *Biochem. J.*, **59,** 433 (1955).

(26) P. S. Chen, T. Y. Toribara, and H. Warner, *Anal. Chem.*, **28,** 1756 (1956).

(27) W. D. Schleuning and H. Fritz, *Methods Enzymol.*, **45,** 330 (1976).

(28) A. C. Gilman, *Proc. Natl. Acad. Sci. USA*, **67,** 305 (1970).

(29) R. V. Tomlinson, H. J. Ringold, M. C. Qureshi, and E. Forchielli, *Biochem. Biophys. Res. Commun.*, **46,** 552 (1972).

(30) M. Glatt, H. Kälin, K. Wagner, and K. Boune, *Agents Action*, **7,** 321 (1977).

(31) I. H. Hall and G. L. Carlson, *J. Med. Chem.*, **19,** 1257 (1976).

(32) T. Y. Shen and C. A. Winter, *Adv. Drug Res.*, **12,** 89 (1977).

(33) H. Bourne, L. Lichtenstein, K. Melmom, C. Henncy, C. Weinstein, and G. Shearer, *Science,* **184,** 19 (1974).

(34) M. Kalimer and K. F. Austen, *Biochem. Pharmacol.*, **23,** 763 (1974).

(35) B. Weiss and W. N. Hait, *Annu. Rev. Pharmacol.*, **17,** 441 (1977).

(36) P. Davis and R. J. Bonney, *Annu. Rep. Med. Chem.*, **12,** 152 (1977).

(37) V. Stefanovich, *Res. Commun. Chem. Pathol. Pharmacol.*, **7,** 537 (1974).

(38) S. H. Ferreira and J. R. Vane, *Annu. Rev. Pharmacol.*, **14,** 57 (1974).

(39) H. E. Paulus and M. W. Whitehouse, *ibid.*, **13,** 107 (1973).

(40) W. L. Smith and W. E. M. Lando, *J. Biol. Chem.*, **21,** 6700 (1971).

(41) G. Weissman, P. Dukor, and R. B. Zurier, *Nature New Biol.*, **231,** 131 (1971).

(42) H. R. Bourne, R. I. Lehrer, M. J. Cline, and K. L. Melmom, *J. Clin. Invest.*, **50,** 920 (1971).

(43) L. J. Ignarro, *J. Immunol.*, **112,** 110 (1974).

ACKNOWLEDGMENTS

Supported by Grant VC-785 from the University of North Carolina Research Council to I. H. Hall.

The synthesis of these agents at Duke University was supported in part by a U.S. Army Research Office Grant.