Table I—Permeability Coefficients Evaluated from Planar Membrane Experiments

Solute	Permeability Coefficient (30°), cm/sec × 10^9
Urea	440
Potassium thiocyanate	21.9
Cesium chloride	3.76
Rubidium chloride	2.84
Potassium chloride	2.37
Sodium chloride	2.19
Lithium chloride	1.77

viscosity at the time of preparation on the species of solute enclosed in the inner aqueous phase. For example, potassium chloride shows a much higher viscosity than potassium thiocyanate. Although it was suggested[10] that potassium thiocyanate prevents formation of W/O/W to give a lower viscosity, this can also be explained in terms of the permeability difference between the two salts. Since potassium thiocyanate, which is more permeable than potassium chloride, would contribute to the osmotic pressure of the inner aqueous phase less than potassium chloride, there would be less movement of water from the outer to the inner aqueous phase, thus giving a lower viscosity.

Table I lists the permeability coefficients calculated for some solutes from the fluxes determined in the planar membrane experiment. The sequence of permeability coefficient values in the table is identical with that obtained in the viscometry of the W/O/W emulsion. Furthermore, the absolute values of the permeability coefficient for sodium chloride and urea are in good agreement with those obtained with phospholipid bilayer membranes (15, 16). The sequence for alkali chlorides is also identical with that obtained with the lipid bilayers (15).

It would be reasonable to conclude that solutes can permeate the oil layer of vesicles in the W/O/W emulsion, probably due to a large total surface area of the vesicles, even though there is a considerable difference in composition between the oil layer and the planar membrane. Water would act as the carrier of solutes across the oil layer, presumably in the solubilized form.

Matsumoto *et al.* (1) proposed to measure the yield of W/O/W emulsion by a dialysis method. That is, when the emulsion containing a solute in the inner aqueous phase is dialyzed against distilled water, the concentration of the solute in the water should correspond to the yield. However, if the solute can permeate the oil layer of vesicles in the emulsion, there is no way to distinguish the solute diffused from the inner aqueous phase from the solute that leaked out as a result of vesicle destruction. The dialysis method cannot give correct yield. Moreover, if a high solute concentration in the water and a low emulsion viscosity are found (as in the case where a highly permeable solute is used) it could be incorrectly concluded that the solute has prevented the formation of a W/O/W emulsion.

REFERENCES

(1) S. Matsumoto, Y. Kita, and D. Yonezawa, *J. Colloid Interface Sci.*, **57,** 353 (1976).

(2) S. Matsumoto, M. Kohda, and S. Murata, *ibid.*, **62,** 149 (1977).

(3) Y. Kita, S. Matsumoto, and D. Yonezawa, *J. Chem. Soc. Jpn.*, **1977,** 748.

(4) C. J. Benoy, L. A. Elson, and R. Schneider, *Br. J. Pharmacol.*, **45,** 135 (1972).

(5) T. Takahashi, M. Mizuno, Y. Fujita, S. Ueda, B. Nishizawa, and S. Majima, *Gann*, **64,** 345 (1978).

(6) T. Takahashi, U. Satoshi, K. Kono, and S. Majima, *Cancer*, **38,** 1507 (1976).

(7) T. Takahashi, K. Kono, and T. Yamaguchi, *J. Exp. Med.*, **123,** 235 (1977).

(8) M. Hashida, M. Egawa, S. Muranishi, and H. Sezaki, *J. Pharmacokinet. Biopharm.*, **5,** 225 (1977).

(9) S. Matsumoto, Y. Ueda, Y. Kita, and D. Yonezawa, *Agr. Biol. Chem.*, **42,** 739 (1978).

(10) H. Fukuda and M. Tanaka, Japanese pat. 134,029 (1977).

(11) Y. Kita, S. Matsumoto, and D. Yonezawa, *J. Colloid Interface Sci.*, **62,** 87 (1977).

(12) S. Matsumoto and M. Kohda, *ibid.*, **73,** 13 (1980).

(13) S. Matsumoto, T. Inoue, M. Kohda, and K. Ikura, *ibid.*, **77,** 555 (1980).

(14) Y. Kita, S. Matsumoto, and D. Yonezawa, *J. Chem. Soc. Jpn.*, **1978,** 11.

(15) V. K. Miyamoto and T. E. Thompson, *J. Colloid Interface Sci.*, **25,** 16 (1967).

(16) R. E. Wood, F. P. Wirth, Jr., and H. E. Morgan, *Biochim. Biophys.* **163,** 171 (1968).

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Ion Chromatographic Determination of the Principal Inorganic Ion in Bulk Antibiotic Salts

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Received May 13, 1981, from *Pfizer Inc., Quality Control Division, Groton, CT 06340.* Accepted for publication July 28, 1981. *Present address: IBM Instrument Inc., Orchard Park, P.O. Box 332, Danbury, CT 06810.

Abstract □ Two ion-chromatographic procedures were developed for determining the principal inorganic ions in bulk antibiotic salts. The anion system enables sulfate determination in sulfate salts of polymyxin, neomycin, streptomycin, and dihydrostreptomycin, with typical analysis times of 8 min/sample and a relative precision of ±2% (95% confidence interval). The cation system is applicable to sodium or potassium analysis in the respective penicillin salts in 9–16 min with a relative precision of ±2.5%. These methods offer speed, specificity, and easy sample preparation compared with traditional assays.

Keyphrases □ Ion chromatography—determination of inorganic ion in bulk antibiotic salts □ Antibiotics—determination of inorganic ion in salts using ion chromatography □ Inorganic ion—determination in salts of various antibiotics by ion chromatography

Bulk antibiotic salts can be characterized by the determination of their principal components for the purposes of quality assurance. An accurate assay is required for both the pharmacologically active organic ion and the inorganic counterion. For the organic moiety, many specific chromatographic assays (1) are applicable to bulks. However,

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Table I—Instrument Conditions for the Ion Chromatographic Analysis of Antibiotic Bulks

	Anion System	Cation System
Columns	3 × 150-mm anion separator	3 × 150-mm cation precolumn
		6 × 250-mm cation separator
	6 × 250-mm anion suppressor	9 × 250-mm cation suppressor
Mobile phase	0.003 *M* $NaHCO_3$/0.0024 *M* Na_2CO_3	0.005 *N* HNO_3
Flow rate	2.25 ml/min	3.0 ml/min
Pressure	400–600 psi	360–580 psi
Detector sensitivity	100 μMHO	10 μMHO
Regeneration solution	1 *N* H_2SO_4	1 *N* NaOH
Regeneration flow rate	2.25 ml/min	2.25 ml/min

Table II—Sample Preparation for Inorganic Ion Analysis

Ion	Antibiotic	Target Weight, mg	Sample Concentration Injected, g/ml	Typical Ion Concentrations, g/ml
K^+	Potassium benzyl penicillin	950	285	30
	Potassium phenoxymethyl penicillin	990	297	30
Na^+	Monosodium indanyl carbenicillin monohydrate	1000	200	9
	Sodium carbenicillin	1000	200	11
SO_4^{2-}	Neomycin sulfate	25	30	7
	Dihydrostreptomycin sulfate	50	60	12
	Streptomycin sulfate	50	60	12
	Polymyxin sulfate	50	60	10

for the inorganic ion portion of the salt, only the traditional volumetric, gravimetric, or colorimetric methods are available (2). The classical assays are generally time consuming, cumbersome, and lack specificity.

The present study reports a rapid and specific inorganic analysis technique which is applicable to a variety of antibiotic salts. The technique was termed ion chromatography by Small (3), in reporting the use of a mobile phase suppressor column as part of an ion-exchange system with conductivity detection. A comparison between suppressed and nonsuppressed ion chromatography and the development of ion chromatography were discussed in a recent review (4). Suppressed ion chromatography was chosen for bulk antibiotic analysis because the conductivity detector response is independent of the counterion species. A standard set of conditions applies to the analysis of various anions or cations and to a variety of sample types. Ion chromatography is specific for the ion of interest, and sample preparation only involves dissolving the salt in the mobile phase. Analysis times are generally shorter than those of traditional assays, and the methodology can be automated.

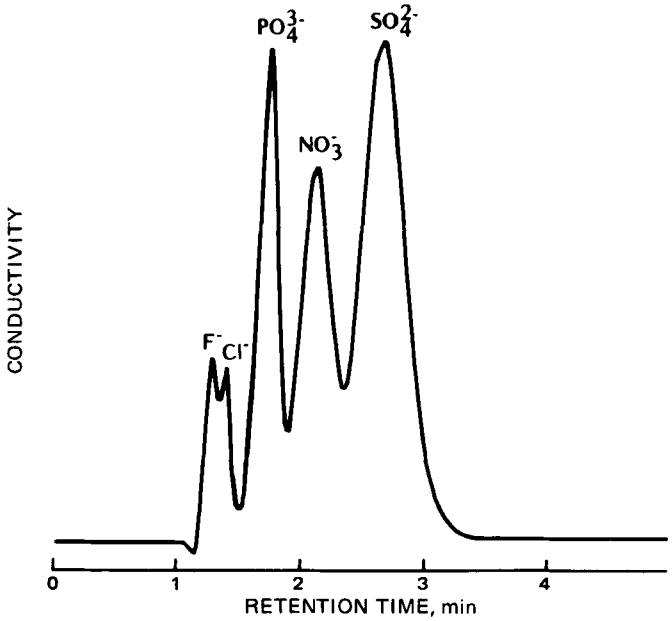

Figure 1—*Chromatogram demonstrating the resolution of standard anions utilizing a 3 × 150-mm separator column.*

Table III—Reproducibility of Sulfate Analysis by Ion Chromatography

Antibiotic	SO_4^{2-}, %[a]	Relative Precision, %[b] Manual	Automated
Polymyxin sulfate	17.12 ± 0.10	1.6	2.5
Streptomycin sulfate	18.12 ± 0.04	0.6	2.3
Dihydrostreptomycin sulfate	18.79 ± 0.07	1.0	0.8
Neomycin sulfate	27.73 ± 0.17	0.6	3.0

[a] Confidence interval, 1 *SD*. [b] Confidence interval, 95%.

Table IV—Reproducibility of Cation Analysis by Ion Chromatography

Cation	Anion	Cation, %[a]
K^+	Benzyl penicillin	10.53 ± 0.26
K^+	Phenoxymethyl penicillin	10.20 ± 0.24
Na^+	Indanyl carbenicillin	4.57 ± 0.12
Na^+	Carbenicillin monohydrate	5.40 ± 0.14

[a] Confidence interval, 95%.

EXPERIMENTAL

Apparatus—A commercially available ion chromatograph[1] containing a 100-μl loop-type injector and conductivity detector with a dual pen strip chart recorder[2] or computing integrator[3] was used. The autosampler[4] employed a 50-μl injector loop connected after the manual injection loop and before the first separator column. The prepacked chromatographic columns were obtained commercially[5], and 0.22-μm disposable filters were used to treat sample solutions[6].

Materials—Reagent grade inorganic salts were sufficiently pure to be used as obtained. All water was passed through a mixed bed deionizer and an activated carbon unit[7].

Method—The analysis conditions and instrument settings are shown in Table I. The bulk antibiotic was dried, and a sample weighing approximately the amount specified in Table II was dissolved and diluted with mobile phase to prepare the sample stock solutions. A working solution was prepared by diluting the stock solution with mobile phase to obtain the final sample concentrations (Table II).

The chromatographic system required equilibration for ~1 hr, until

[1] Dionex model 14 ion chromatograph.
[2] Honeywell dual channel/dual pen variable input recorder.
[3] Spectra Physics model 4100 computing integrator.
[4] Micromeritics model 725 autosampler.
[5] Dionex Corp., Sunnyvale, Calif.
[6] Millipore Millex-GS 0.22 μm.
[7] Continental water conditioning system models 350 and 350A.

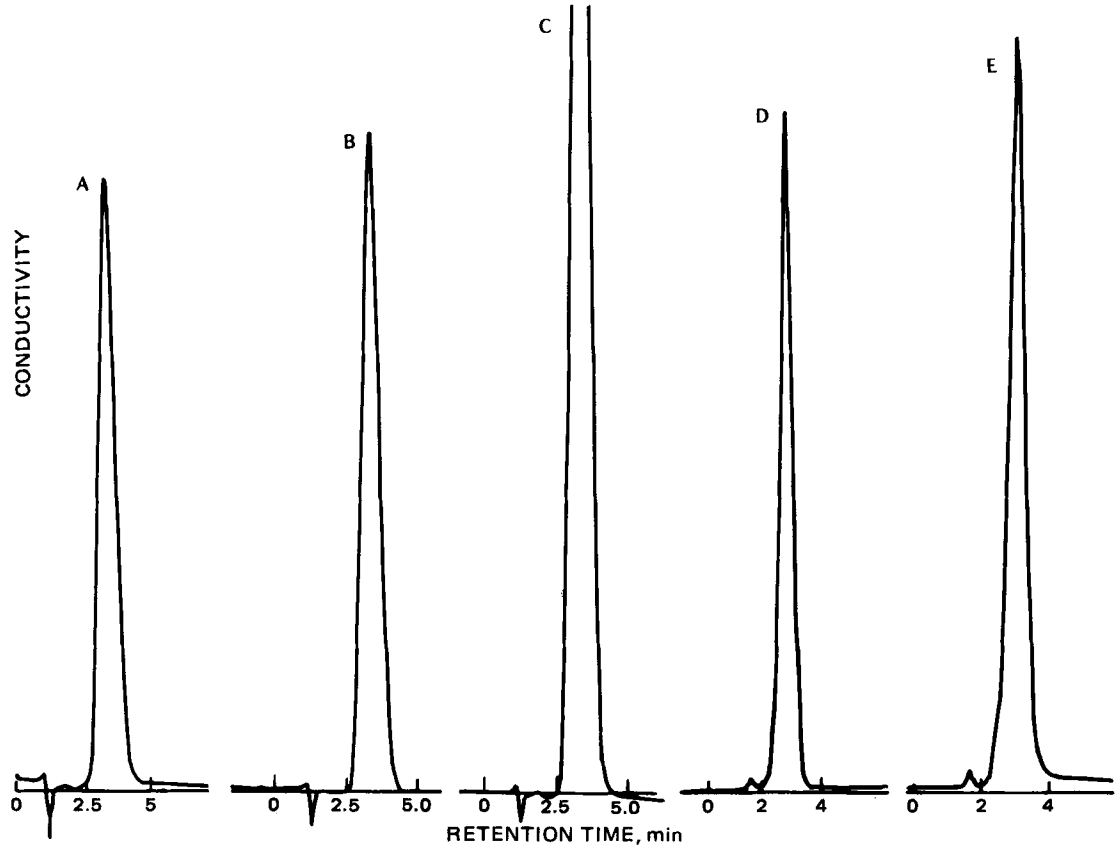

Figure 2—*Chromatograms obtained during sulfate analysis. The sodium sulfate standard chromatogram (A), is indistinguishable from the sulfate component of the organic salts neomycin (B), dihydrostreptomycin (D), and polymyxin (E), and from the spike of sodium sulfate into neomycin sulfate (C).*

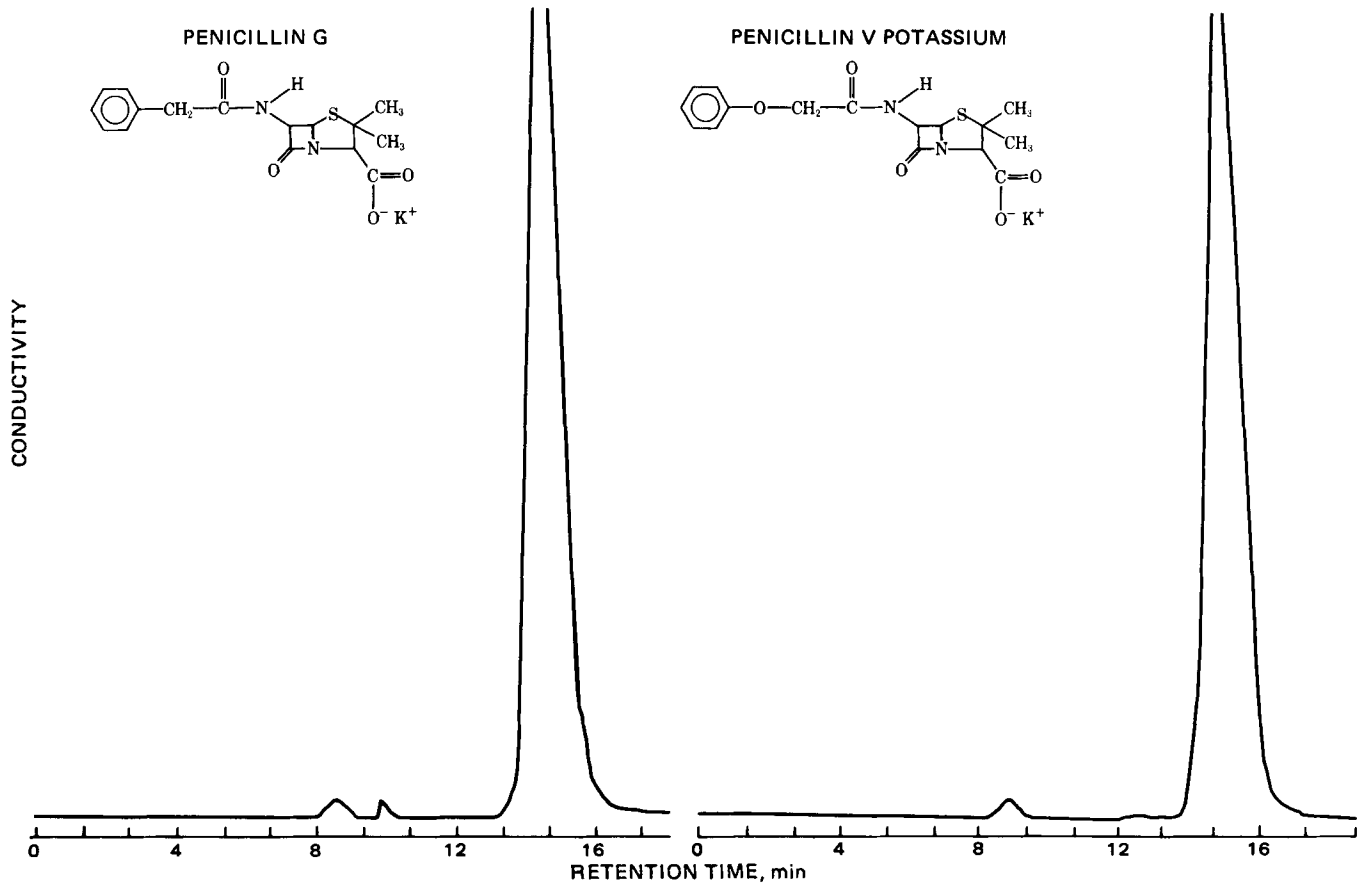

Figure 3—*Chromatograms demonstrating potassium determination in potassium phenoxymethyl penicillin and potassium benzyl penicillin.*

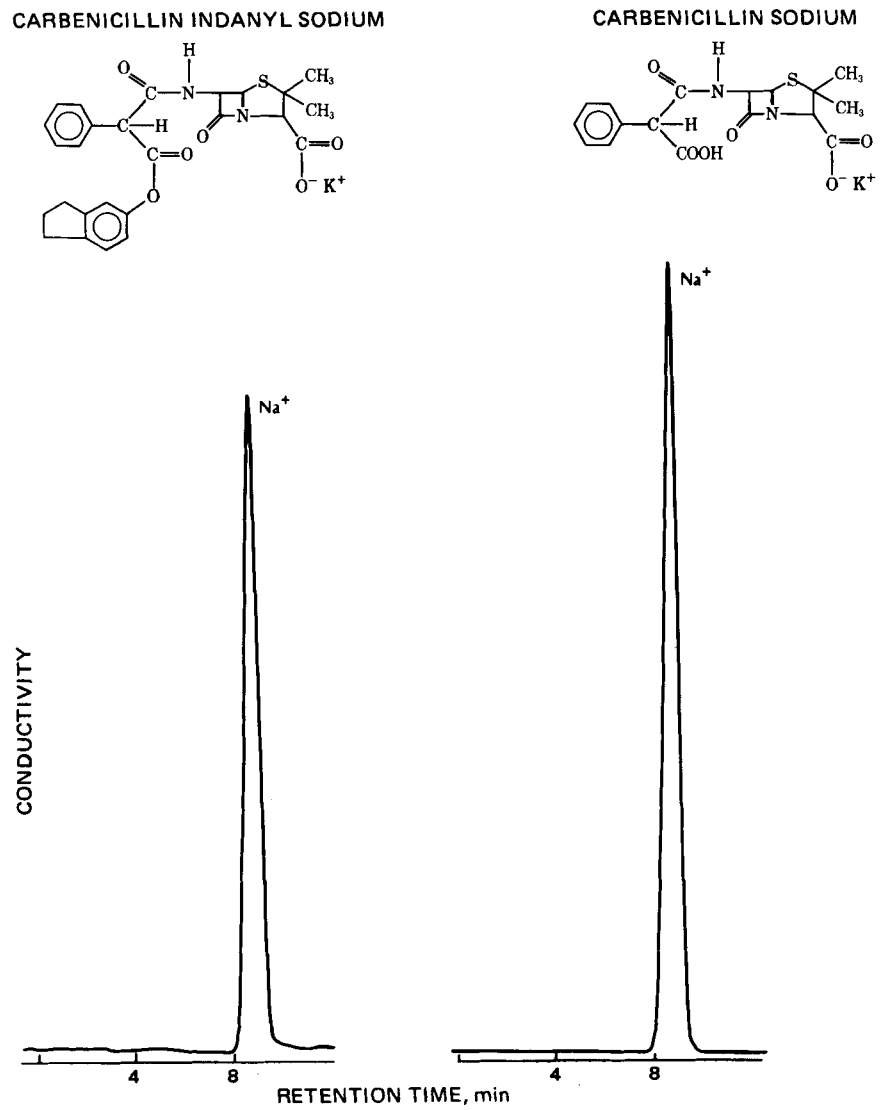

Figure 4—*Chromatograms demonstrating sodium determination in monosodium indanyl carbenicillin (MIC) and monosodium carbenicillin (MSC).*

a stable baseline was obtained. Duplicate injections of the working standard were made, and the peak retention time and response reproducibility were compared with previously determined values in order to check the suitability of the instrument system.

Sample solutions were injected through the disposable filter into the ion chromatograph or autosampler vial. For the autosampler, sample and rinse solutions were alternately injected.

After 5–6 samples were injected, the system was recalibrated by reinjecting the working standard. The sample concentrations can be calculated using either peak height or peak area measurement techniques.

RESULTS AND DISCUSSION

Anion Analysis—The anion system was identical with the systems generally recommended for common anion analysis, (5, 6) except that a short (3 × 150 mm) separator anion exchange column was employed. This short separator column enabled rapid analysis with adequate resolution (Fig. 1). In bulk antibiotic salts, potentially interfering ions are present in only small amounts, thereby minimizing the need for large separator columns and long analysis times.

In the event that simultaneous analyses of the major ion and some trace ion are desired, a longer separator column could be employed. For example, a system that can analyze both 0.1–0.4% bisulfite and 13–38% sulfate in a sulfate salt was developed using both a 3 × 150-mm and a 3 × 500-mm separator column. The bisulfite eluted with a relative retention time of 0.75 (9 min) to sulfate (12 min). Glycerin (1%) was used to stabilize the bisulfite; the glycerin had no effect on the chromatography.

Typical chromatograms (Fig. 2) obtained during sulfate analysis show that the sulfate peak in the antibiotic salt was indistinguishable from that of the sodium sulfate standard.

Linearity—Standards containing 5–20 μg/ml sulfate gave a linear response by both peak height and peak area computation. This range encompasses the theoretical sulfate content for the antibiotic salts, which are 26.9% for neomycin, 19.7% for streptomycin, 19.7% for dihydrostreptomycin, and 16.6% for polymyxin.

Reproducibility—The assay precision was determined by 5–6 replicate analyses of the same antibiotic lot. The precision values (Table III) include variations due to weighing and sample preparation. The manual analyses exhibited better precision than the automated analyses; although the precision of the automated method was adequate, no attempt was made to improve its precision. The precision of the automated method became unacceptable if a sample rinse was not employed to prevent carry over of the sulfate from the previous injection. The automated ion chromatograph was successfully employed for the analysis of all of the sulfated antibiotics in overnight runs.

Equivalency—Two classical methods for sulfate determination can be applied to sulfate analysis in antibiotics: the traditional barium gravimetric method (7) and a volumetric (8) method. The latter has been proposed for incorporation into the *European Pharmacopeia* monograph for polymyxin sulfate. To compare these methods and the ion chromatography technique, various lots of polymyxin sulfate were assayed by both the classical and ion chromatographic methods. For eight lots assayed by both gravimetric and ion chromatography, the average relative difference was −0.6 ±1.1% (confidence interval: 1 *SD*). Similarly, for 14 lots examined by the volumetric and ion chromatographic methods, the difference was −0.3 ±2.7%. The data indicate no apparent bias between

the methods. In both cases, the ion chromatographic method is more rapid and involves less sample manipulation than the traditional assays.

Cation Analysis—An ion chromatographic method, similar to the method reported for the determination of cations in ambient air aerosols (9), was successfully applied to the analysis of various penicillins (Figs. 3 and 4). The cation system, like the anion system, is independent of the nature of the counter-ion.

Linearity—Standards containing 5–15 μg/ml sodium and 10–40 μg/ml potassium gave linear responses by both peak height and peak area computation (Figs. 3 and 4). These ranges encompass the theoretical cation content for the antibiotic salts. The stoichiometric potassium level is 10.07% in penicillin V potassium (potassium phenoxymethyl penicillin) and 10.50% in penicillin G (potassium benzyl penicillin). Theoretical sodium levels in the sodium salts are 4.45% in carbenicillin indanyl sodium and 5.49% in carbenicillin sodium monohydrate. The sample concentrations (Table II) were chosen to give a similar response for both cations so that samples could be batched.

Reproducibility—Typical precisions of the ion chromatographic cation determination are shown in Table IV. These experiments include the complete replicate analysis of the same bulk lot.

Equivalency—No compendial requirement exists for the potassium content of the penicillins; however, both assays agreed well with the theoretical values.

The ion chromatographic sodium analysis was compared with sodium content determined by ashing six different antibiotic lots in the presence of sulfuric acid and measuring the remaining sodium sulfate gravimetrically. For carbenicillin sodium monohydrate, the relative difference was $-1.0 \pm 3.3\%$ (confidence interval: 1 *SD*). Carbenicillin indanyl sodium by ion chromatography was $3.9 \pm 4.3\%$ greater than the analysis by the residue on ignition method. Since both differences are smaller than the confidence interval, there was no apparent bias between methods.

REFERENCES

(1) R. K. Gilpin, *Anal. Chem.*, **51**, 257R (1979).

(2) "The United States Pharmacopeia XX–National Formulary XV," United States Pharmacopeial Convention, Rockville, Md., 1980.

(3) H. Small, T. Stevens, and W. Bauman, *Anal. Chem.*, **47**, 1801 (1975).

(4) C. A. Pohl and E. L. Johnson, *J. Chromatogr. Sci.*, **18**, 442 (1980).

(5) J. D. Mulik, E. Estes, and E. Sawicki, in "Ion Chromatographic Analysis of Environmental Pollutants," E. Sawicki, J. D. Muilk, and E. Wittgenstein, Eds., Ann Arbor, Mich., 1978, chap. 4.

(6) T. S. Long and A. L. Reinsvold, *Jt. Conf. Sens. Environ. Pollut. (Conf. Proc.), 4th*, 1977 (Pub. 1978), 624.

(7) B. J. Heinrich, M. D. Grimes, and J. E. Puckett "Treatise on Analytical Chemistry," I. M. Kolthoff, P. J. Elving, and E. B. Sandell, Eds., vol. 7, part II, New York, N.Y., 1961, p. 103.

(8) "European Pharmacopeia," vol. II, 1971, p. 344; Proposed Revision Polymyxin Sulfate Monograph, PA/PH/EXP. 7/T (78) 26, 1st rev.

(9) "Ion Chromatography–A New Analytical Technique for the Assay of Sulfate and Nitrate in Ambient Aerosols," National Bureau of Standards Special Publication 464, U.S. Government Printing Office, Washington, D.C., 1977.

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Antitumor Agents XLV: Bisbrusatolyl and Brusatolyl Esters and Related Compounds as Novel Potent Antileukemic Agents

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Received April 6, 1981, from the **Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514* and the ‡Wellcome Research Laboratories, Burroughs Wellcome Co., Research Triangle Park, NC 27709. Accepted for publication July 15, 1981.

Abstract □ A series of new bisbrusatolyl and brusatolyl esters and related compounds were synthesized and tested for *in vivo* antileukemic activity against a quassinoid sensitive strain of P-388 lymphocytic leukemia in BDF_1 mice. The bisbrusatolyl malonate, succinate, glutarate, adipate, and sebacate were as active or more active than brusatol. The C-3 esters of brusatol and bruceantin were also found to be as active or more active than brusatol or bruceantin in general. The free hydroxyl groups at C-11 and C-12 as well as the enone double bond in ring A of both bisbrusatolyl and brusatolyl esters are required for antileukemic activity. The presence of a double bond in the ester side chain contributes to the enhanced activity of these esters.

Keyphrases □ Brusatol—bisbrusatolyl and bisbrusatolyl esters, synthesis, potential antileukemic agents □ Antitumor agents—potential, bisbrusatolyl and bisbrusatolyl esters, synthesis □ Antileukemic agents—potential, bisbrusatolyl and bisbrusatolyl esters, synthesis, tested against P-388 lymphocytic leukemia

The structural requirements for antineoplastic activity (particularly in the P-388 mouse lymphocytic leukemic system) of quassinoids bruceantin (I), holacanthone, glaucarubolone, 6α-senecioyloxychaparrinone, and related compounds have recently been reviewed (1–6). It was concluded that the Δ^3-2-oxo moiety in ring A, the lactone moiety in ring D, the ester groups at either C-6 or C-15, the methyleneoxy bridge, and the hydroxyl moieties at either C-1 or C-3, and at C-12 are required for biological activity.

The isolation of novel antileukemic glycosides bruceoside-A and bruceoside-B, as well as their subsequent hydrolysis product brusatol (II) (7, 8) provided the opportunity for developing brusatol related compounds into future clinically active anticancer agents. Brusatol is structurally identical to bruceantin (I) [currently in the Phase II clinical trial as an anticancer agent by the National Cancer Institute (9)] except for a slight difference in the C-15 ester side chain. The C-15 ester moiety in I is important for its potent antileukemic activity and probably serves as a carrier group in processes such as membrane transport of the drug into intact cells or complex formation as previously suggested (2, 6, 10). The importance of the ester group which contributes to the enhanced antileukemic activity is also seen in other naturally oc-

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