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SEPARATION OF 6-THIOPURINE DERIVATIVES ON DEAE-SEPHADEX COLUMNS AND IN THE HIGH-PRESSURE LIQUID CHROMATOGRAPH

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SUMMARY

Conditions are described for the separation of 6-thiopurine derivatives of pharmacological interest, both on large columns of DEAE-Sephadex A-25 by a modified procedure of CALDWELL[1] and in the Varian Aerograph LCS-1000 high-pressure liquid chromatograph. It was found that the triethylammonium acetate buffer, pH 4.7, used by CALDWELL caused extensive degradation of 6-thiopurines containing an unsubstituted thiol group, and that this decomposition could be prevented by the addition of 10 m*M* β-mercaptoethanol. Elution profiles are presented for a number of synthetic 6 thiopurine derivatives investigated by these two chromatographic procedures.

INTRODUCTION

This laboratory has long had an active interest in the pharmacology of 6-thiopurines, both as anticancer and as immunosuppressive agents[2]. More recent work has involved a study of the metabolism of [^{35}S]MP* in the transplantable mouse mammary tumor, *Adenocarcinoma 755*. This study led to the investigation of various chromatographic procedures for the separation, identification and quantitation of the acid-soluble, radioactive metabolites of [^{35}S]MP.

The procedure of CALDWELL[1], which employs a large column of DEAE-Sephadex A-25 with consecutive concentration gradients of TEA acetate buffer, pH 4.7, initially presented problems, due to extensive degradation of the [^{35}S]-thiopurine metabolites which occurred during the operation of the column and the subsequent recovery of the individual metabolites. However, it was found that 6-thiopurines could be stabilized in the presence of TEA acetate, pH 4.7, by the addition of 10 m*M* β-ME to this buffer. This technique proved especially useful when it was necessary to isolate sufficient quantities of each radioactive metabolite for definitive characterization.

Another procedure employed extensively in this laboratory involved high-pressure liquid chromatography (LC). With the Varian Aerograph LCS-1000 liquid chromatograph, conditions were developed which, with respect to the major acid-soluble metabolites of [^{35}S]MP, afforded resolution approaching that obtained with CALDWELL's procedure. Once the major radioactive metabolites of [^{35}S]MP

* The abbreviations used in this paper are explained in the section ABBREVIATIONS.

had been identified and their retention times had been determined, high-pressure LC proved to be a rapid and convenient technique for the routine determination of the metabolic pattern of large numbers of tissue extracts.

This paper describes in detail the conditions employed in the above-mentioned chromatographic procedures and presents elution profiles obtained with a number of synthetic 6-thiopurine derivatives which were investigated by these two procedures.

EXPERIMENTAL

Chemicals

DEAE-Sephadex A-25 was purchased from Pharmacia Fine Chemicals. TEA, 2,4-diaminophenol dihydrochloride, and β-ME were products of Eastman Kodak Co. TEA was purified by a 2-h reflux in the presence of 2,4-diaminophenol followed by distillation at atmospheric pressure as described by PARISH[3]. AMP, ADP, ATP, TX, and MMPR were from Sigma Chemical Co. TIMP was obtained from P-L Biochemicals, Inc. Alkaline phosphatase (calf intestine) was a product of Calbiochem. Inorganic ^{35}S-sulfate and ^{35}S-MP (specific activy, 2.0 μCi/μmole) were purchased from Amersham/Searle Corp. MMPRP and TGMP were synthesized from the corresponding ribonucleosides by Drs. LOWRIE M. BEACHAM, III and RICHARD L. MILLER of these laboratories according to the method of IRIE[4]. TXMP was synthesized enzymatically by Dr. RICHARD L. MILLER using the partially purified guanine phosphoribosyltransferase from *Escherichia coli*[5]. TXR was formed by dephosphorylation of TXMP with alkaline phosphatase. MP[6], MPR[7], TG[8], TGR[7], 8-OH-MP[9], TU[10], MMP[6], MTU[11], azathioprine[12] and 8-hydroxyazathioprine[11] were synthesized in this laboratory.

Stability study of 6-thiopurines

The stability of several 6-thiopurines was determined spectrophotometrically during a 4-day incubation in TEA acetate buffer, pH 4.7. Solutions (10 ml) of each thiopurine were prepared in various concentrations of TEA acetate, pH 4.7, both in the presence and absence of 10 m*M* β-ME. Incubations were carried out at room temperature in open test tubes.

Initial ($t = 0$) absorbance values of these thiopurine solutions were in the range of 0.730–1.140 when measured at the following $\lambda_{max.}$ settings: TGMP, 342 nm; TIMP, 322 nm; TU, 349 mn. (Similar studies with TXMP and MMPRP, not presented in detail in this report, used $\lambda_{max.}$ values of 341 and 291 nm, respectively.) The absorbance of each solution was measured daily against an appropriate blank at the $\lambda_{max.}$ determined for each thiopurine.

Initial and final ultraviolet (UV) absorption spectra of each thiopurine solution were recorded with a Beckman Acta III spectrophotometer. Daily absorbance measurements were carried out with a Gilford Model 222 photometer coupled with the monochromator section of a Beckman DU spectrophotometer.

Standard solutions

Fresh solutions of each thiopurine standard were prepared at a concentration of 7–10 m*M*. With the exception of azathioprine, all of the thiopurines were dissolved

in 10 m*M* β-ME. The solution of TXR was acidified to pH 4.5 to inactivate the alkaline phosphatase which was still present. Solutions of AMP, ADP, and ATP were prepared at a concentration of 10 m*M* and were stored at −20°.

DEAE-Sephadex chromatography

DEAE-Sephadex A-25 was hydrated and equilibrated with starting buffer essentially as described by CALDWELL[1]. The resulting slurry of ion exchanger was poured into a 2.5 × 100-cm glass column (Glenco Scientific, Inc.) and allowed to pack by gravity to a height of *ca.* 90 cm. The packed column was then washed with starting buffer at a flow-rate of 100 ml/h (using a Sigmamotor Model T8 finger pump) until the UV absorbance (254 nm) of the column effluent attained a constant value. The sample was applied to the bared top of the column bed with a pipet and was washed down into the column bed with several small portions of starting buffer. Elution was then commenced with Gradient I.

Concurrent with the enlargement of CALDWELL's column dimensions, a modification of the gradient elution scheme was also made. Two columns of DEAE-Sephadex A-25 of the above dimensions were routinely eluted simultaneously with a single gradient-generating apparatus feeding both columns. The mixing chamber consisted of a 9.5 × 30-cm bottle with two outlets at its base. The reservoir was a 2000-ml conical flask and was connected to the mixing chamber by means of a siphon tube. All buffers contained 10 m*M* β-ME. Gradient I was generated from 2000 ml of 0.04 *M* TEA acetate, pH 4.7, (starting buffer) plus 2000 ml of 0.35 *M* TEA acetate, pH 4.7. Gradient II was formed from 2000 ml each of 0.35 and 1.0 *M* TEA acetate, pH 4.7. Gradient III was composed of 2000 ml of 1.0 *M* and 1000 ml of 1.4 *M* TEA acetate, pH 4.7, respectively. Gradient IV consisted of 1000 ml each of 1.4 *M* TEA acetate, pH 4.7, and of 1.4 *M* TEA acetate, pH 4.7, containing 1.0 *M* formate. Gradient V was simply elution with 1.4 *M* TEA acetate, pH 4.7, containing 2.0 *M* formate. Gradients I–V extended to fractions 85, 174, 245, 275, and 305, respectively, in Fig. 2. The flow-rates of both columns were maintained at 100 ml/h with a finger pump. The UV absorbance (254 nm) of the column effluent was recorded, and fractions (22 ml) were collected using a Model UA-2 UV analyzer and a fraction collector from Instrumentation Specialties Co.

The UV absorbing compounds were located among the fractions of column effluent on the recorder paper of the UV analyzer, and the UV absorption spectrum was measured with the fraction containing the highest concentration of each compound. Peak identities in Fig. 2 are based both upon the UV absorption spectrum of each peak and upon the elution positions of these standards when chromatographed individually under identical conditions. In addition, the elution positions of many of these compounds are known from the characterization of radioactive metabolites of [^{35}S]MP which were separated on identical columns. The elution position of $^{35}SO_4^{2-}$ in Fig. 2 was established by counting 1.0-ml aliquots of each fraction in 10 ml of scintillation fluid. This elution position was verified with tumor extracts by the addition of unlabeled SO_4^{2-} to the sample and subsequent precipitation of the eluted SO_4^{2-} with $BaCl_2$.

High-pressure liquid chromatography

A Varian Aerograph LCS-1000 liquid chromatograph was used. In addition

to the standard UV detector (254 nm) supplied with the instrument, a second UV detector (Model 1280 from Laboratory Data Control) was often used simultaneously to monitor the column effluent at either 280 or 350 nm. The cylindrical flow cells were 1 mm in diameter and had a 10-mm pathlength. A two-pen, 10-mV Honeywell Electronik 194 recorder was employed. The column, supplied by Varian Aerograph, was 1.0 mm I.D., 3 m in length and was packed with a pellicular anion-exchange resin (batch PA-38). When desired, timed fractions of the column effluent were collected directly into liquid scintillation vials using a rotary fraction collector (Rinco Instrument Co.) modified slightly to hold the vials.

The low- and high-concentration eluents were 0.03 and 4.0 *M* ammonium acetate, pH 4.7, respectively. The starting volume of low-concentration eluent in the mixing chamber was 50 ml. The column flow-rate was 24 ml/h and the gradient flow-rate was 12 ml/h. A 15-min gradient delay was employed. The oven temperature was maintained at 70°. Two consecutive 15-min purges with low-concentration eluent were required to equilibrate the column between runs; the mixing chamber was drained and refilled with low-concentration eluent between these two purges. Samples were introduced into the liquid chromatograph with a 20-μl syringe (Glenco Scientific, Inc.).

Peak identities in Fig. 3 are based both upon the UV absorption spectrum of each peak (collecting 1-min fractions) and upon the retention times determined with the individual standards under identical conditions. The retention time of $^{35}SO_4^{2-}$ was determined by counting 10-μl aliquots of each 1-min (*ca.* 0.4 ml) fraction of the column effluent in 10 ml of scintillation fluid. The retention time of SO_4^{2-} was verified in a tumor extract by adding unlabeled SO_4^{2-} to the sample and monitoring the column effluent for the appearance of a precipitate with $BaCl_2$.

Other procedures

Perchloric acid extracts were prepared from ^{35}S-MP-treated tumors by the method of NELSON *et al.*[13] and were made 10 m*M* with respect to β-ME at the time of neutralization with KOH.

RESULTS AND DISCUSSION

Chromatography of 6-thiopurines on DEAE-Sephadex

CALDWELL[1] and PATERSON AND WANG[14] have reported the apparently successful application of the column chromatographic procedure, employing DEAE-Sephadex with consecutive concentration gradients of TEA acetate buffer, pH 4.7, to metabolic studies involving 6-thiopurines. However, severe difficulties were encountered in our initial attempts to utilize this procedure for the separation of the acid-soluble metabolites of ^{35}S-MP: peaks of radioactivity were poorly resolved and five out of six of the major radioactive peaks, which were subsequently recovered, analyzed to be $^{35}SO_4^{2-}$. The only major metabolite which appeared to survive this chromatographic procedure intact was MMPRP.

A subsequent spectrophotometric study established that several representative 6-thiopurines are unstable in TEA acetate, pH 4.7 under the conditions employed. The data presented in Fig. 1 demonstrate the extensive and rapid (relative to the time required to complete elution of a DEAE-Sephadex column) degradation of

TU, TIMP, and TGMP during incubation in two different concentrations (0.92 and 3.69 *M*) of TEA acetate, pH 4.7, as well as the protective effect of β-ME. The addition of 10 m*M* β-ME to 3.69 *M* TEA acetate conferred nearly complete stability (*i.e.* less than 5 % decrease in absorbance after 4 days) on the 6-thiopurines which were examined. (A less detailed study revealed that TXMP is similarly unstable in 1.0 *M* TEA acetate, pH 4.7, whereas MMPRP was completely stable in this buffer even in the absence of β-ME.) The higher concentration (3.69 *M*) of TEA acetate was examined here in view of the likely exposure of 6-thiopurine metabolites to similar or higher concentrations of this salt during the lengthy lyophilization which follows chromatographic separation of the metabolites.

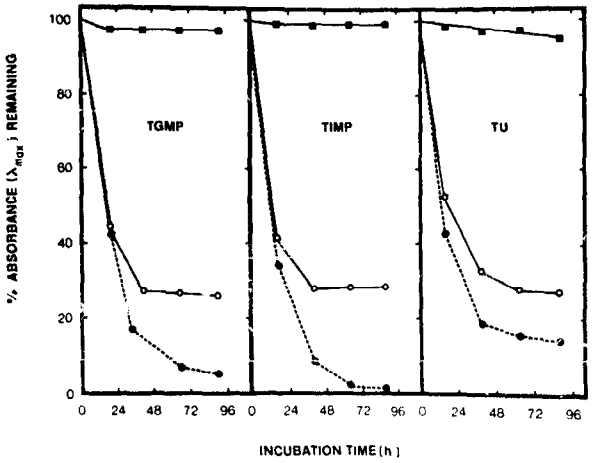

Fig. 1. Kinetics of degradation of 6-thiopurines in TEA acetate buffer, pH 4.7. Solutions (10 ml) of each thiopurine were prepared in: ○—○, 0.92 *M* TEA acetate; ●—●, 3.69 *M* TEA acetate; and ■—■, 3.69 *M* TEA acetate containing 10 m*M* β-ME; and were incubated for 4 days at room temperature in open test tubes. The absorbance of each solution was measured daily against an appropriate blank at the $\lambda_{max.}$ determined for each thiopurine.

The nature of this degradation of 6-thiopurines in TEA acetate has not been characterized in detail. However, spectral examination of the products formed during incubation in this buffer indicated that TIMP, TGMP and TXMP had been converted to their corresponding 6-oxypurine analogues (presumably liberating SO_4^{2-}), whereas TU appeared to be converted to another species of 6-thiopurine exhibiting an absorption maximum at 324 nm. With the exception of TU, which exhibited an 11 % decrease in absorbance, all of the above-studied 6-thiopurines were completely stable during a 4-day incubation in 1.0 *M* ammonium acetate, pH 4.7, in the absence of a reducing agent.

On the basis of the results shown in Fig. 1, all subsequent chromatography of 6-thiopurines on DEAE-Sephadex has been carried out with the inclusion of 10 m*M* β-ME in the gradients of TEA acetate. With this minor but important modification, CALDWELL's chromatographic procedure has yielded excellent and highly reproducible resolution of the radioactive metabolites of [^{35}S]MP. Furthermore, the radioactive metabolites which were isolated by this slightly modified procedure were found to be sufficiently stable for subsequent characterization.

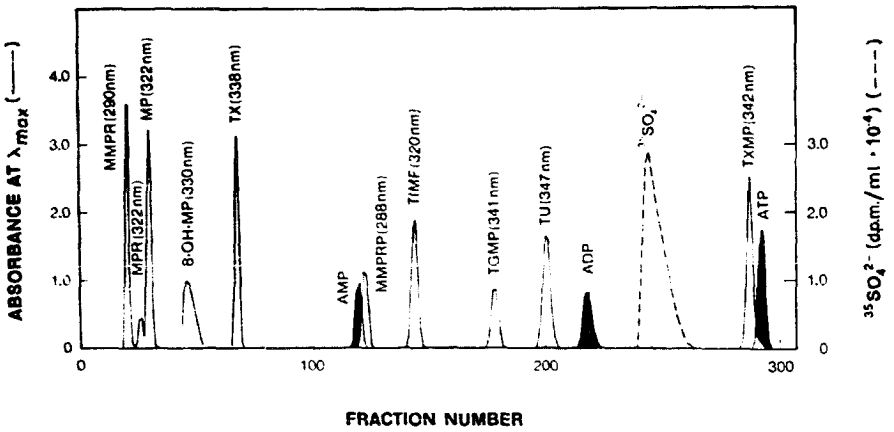

Fig. 2. Chromatography of 6-thiopurine standards on DEAE-Sephadex columns in the presence of 10 m*M* β-ME. The details of this experiment are presented in the EXPERIMENTAL section. The adenine nucleotides (AMP, ADP, ATP) were co-chromatographed and are represented by the blackened peaks. The absorbance values (solid line) plotted in this figure were measured at the λ_{max}, which was determined with each peak. The identity of each peak as well as its λ_{max} is included in the figure. Sample: 3.0 ml of a solution containing 6-thiopurine standards (*ca.* 0.1–0.9 m*M* in each), $^{35}SO_4^{2-}$ ($6.4 \cdot 10^5$ d.p.m.), and adenine nucleotides (*ca.* 0.5–0.9 m*M* in each).

As an example of the quality of resolution which has been experienced routinely with this procedure, the elution profile of a set of synthetic 6-thiopurine standards is presented in Fig. 2. The adenine nucleotides (AMP, ADP, ATP) were co-chromatographed in this experiment to serve as convenient reference compounds, and their elution positions are indicated by the use of blackened peaks. All of the bases and ribonucleosides of the 6-thiopurines which have been examined elute prior to AMP with the exception of TXR and TU: the former compound (not included in the set of standards in Fig. 2) elutes just prior to TGMP, while the latter compound elutes shortly before ADP. The ribonucleoside 5'-monophosphates of the 6-thiopurines which have been examined elute between AMP and ATP in the following order: MMPRP, TIMP, TGMP, TXMP. SO_4^{2-} elutes in the nucleotide region, following ADP. From standards chromatographed on other occasions it is known that TGR elutes shortly after MP, while MTU co-elutes with MP. Azathioprine (Imuran) and its metabolite 8-hydroxyazathioprine, when chromatographed in the absence of β-ME (which causes the rapid breakdown of azathioprine by nucleophilic attack at the 5-carbon of the 1-methyl-4-nitroimidazole ring), also co-elute from this column with MP[15]. Preliminary analysis of a tumor extract containing acid-soluble metabolites of ^{35}S-TG suggests that TGTP elutes considerably later than ATP and requires 2 *M* formate in 1.4 *M* TEA acetate to effect elution. With the exception of TXR, the set of standards chromatographed in Fig. 2 includes all of the major metabolites of ^{35}S-MP which have been identified in tumor extracts. The recovery of these thiopurine standards from the DEAE-Sephadex column was essentially quantitative (88–102%). The cause of the broad peaks observed with 8-OH-MP and SO_4^{2-} is not understood; in numerous other experiments these compounds eluted reproducibly as sharp, symmetrical peaks.

CALDWELL[1] had previously identified the relative elution positions of three

of these thiopurines (MMPRP, TIMP and TXMP). The present results are in agreement with this earlier work and define the behavior of a number of other 6-thiopurines of pharmacological interest in this chromatographic system. Neutralized perchloric acid extracts from as much as 30 g of tissue have been successfully chromatographed on these large columns of DEAE-Sephadex (described in the EXPERIMENTAL section) without significant loss of resolution.

High-pressure liquid chromatography of 6-thiopurines

To date, virtually all of the work which has been reported concerning the separation of 6-thiopurines by high-pressure LC has been performed by BROWN[16] and her colleagues[17,18]. BROWN's initial report[16] on the separation of nucleotides by high-pressure LC included the retention times of MMPRP and TGMP relative to the other nucleotides which were investigated. SCHOLAR *et al.*[17] additionally identified the elution position of TIMP in their system. More recent work[18] from the same laboratory established the retention times of TGR, TG, TU, TGDP, and TGTP and utilized high-pressure LC to quantitate several of these metabolites of [^{35}S]TG which were extracted from drug-treated tumor cells. All three of these studies of the high-pressure LC of 6-thiopurine derivatives employed the same concentration gradient, linear in both KH_2PO_4 and KCl, according to the standardized conditions described by BROWN[16].

In the present studies, the same type of instrument (Varian Aerograph LCS-1000 liquid chromatograph) was used, and an alternative gradient elution scheme was devised both for nucleotides in general and for 6-thiopurine derivatives in particular. The essence of this system is a linear gradient (0.03–4.0 *M*) of ammonium acetate (pH 4.7), which is commenced after a 15-min gradient delay. At pH 4.7–4.9, ammonium acetate was found to be superior to ammonium formate, ammonium propionate, lithium acetate, and TEA acetate for the separation of metabolites of [^{35}S]MP.

In all of these studies the starting volume of low-concentration eluent in the mixing chamber was 50 ml, the oven temperature was held constant at 70° and the column flow-rate was maintained at 24 ml/h. The UV absorbance of the column effluent was monitored at 254 nm and often, in addition, at a second wavelength (either 280 or 350 nm). The availability of a UV detector operating at 350 nm renders the high-pressure liquid chromatograph more versatile in this type of study since the 6-thiopurines absorb light maximally in the 290–350 nm range, where most naturally occurring purines and pyrimidines are transparent.

As an illustration of the resolution attainable with this gradient system, the elution profile of a number of synthetic 6-thiopurine standards is presented in Fig. 3. As in Fig. 2 above, the adenine nucleotides (AMP, ADP, ATP) were co-chromatographed with the thiopurines to serve as reference compounds, and their peaks are blackened to allow their ready location. The chromatographic characteristics of the thiopurine standards in the high-pressure liquid chromatograph differed from those described above with the DEAE-Sephadex column in only three notable respects: (1) TXR eluted after TGMP in the liquid chromatograph; (2) SO_4^{2-} eluted prior to ADP in the liquid chromatograph; (3) relative to the adenine nucleotides, the thiopurines exhibited a greater affinity towards the pellicular anion-exchange resin than towards DEAE-Sephadex. The set of 6-thio-

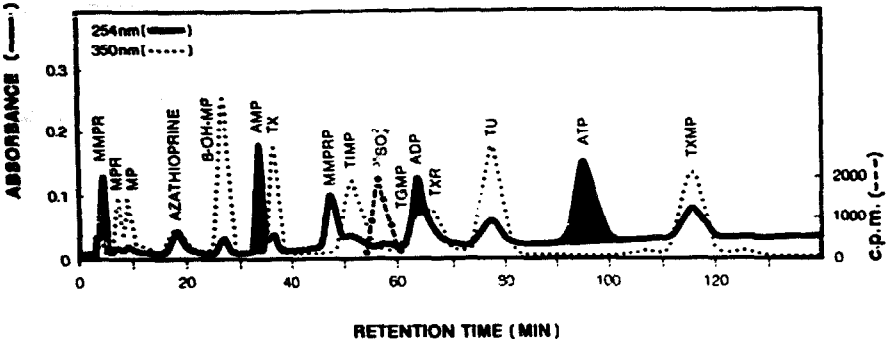

Fig. 3. Separation of 6-thiopurine standards in the high-pressure liquid chromatograph. The adenine nucleotides (AMP, ADP, ATP) were co-chromatographed and are represented by the blackened peaks. Starting volume, 50 ml; flow-rates, 24 and 12 ml/h; eluents, 0.03 and 4.0 *M* ammonium acetate, both at pH 4.7; oven temperature, 70°; gradient delay, 15 min; sample, 15 μl of a solution containing 6-thiopurine standards (*ca.* 0.2–1.6 m*M* in each), $^{35}SO_4^{2-}$ ($7.0 \cdot 10^4$ d.p.m.), and adenine nucleotides (*ca.* 0.3–0.9 m*M* in each); UV range, 0.16 a.u. (absorbance unit) for both photodetectors (254 and 350 nm).

purine standards chromatographed in Fig. 3 represents all of the major metabolites of [^{35}S]MP in tumor extracts and, in addition includes the immunosuppressive drug azathioprine. From other chromatograms it is known that TGR and TC elute coincidentally with MPR and MP, respectively, and that both MTU and MMP exhibit the same retention time as MP. Based upon a preliminary analysis of a tumor extract containing metabolites of ^{35}S-TG, TGTP elutes from the liquid chromatograph considerably later than TXMP and requires a much steeper gradient (*e.g.*, starting at GTP) to hasten its elution. Recovery of these thiopurine standards from the accessory port of the liquid chromatograph was nearly quantitative (88–99%), provided that the sample volume injected into the instrument was not greater than 15 μl.

The sensitivity and utility of this analytical procedure is illustrated by the following considerations. With a range of 0.04 a.u. on the UV detector operating at 350 nm, concentrations of TU, TXMP, TGMP, and TIMP of 250, 430, 730, and 1340 μM, respectively, in a 15-μl sample yield a full-scale signal on the 10-in. recorder chart. Extracts can be readily concentrated 5-fold relative to the original weight of tissue to increase the sensitivity of this method proportionately. Finally, with the low level of noise present, a peak extending only 1/10 full-scale can be conveniently and accurately measured by means of a planimeter. Thus, tissue concentrations of these thiopurines as low as 5–27 μM can be quantitated solely on the basis of their optical properties without recourse to methodology involving radioisotopes. MMPRP, which exhibits a UV absorption maximum at 292 nm, is not detectable at 350 nm. This latter compound is best monitored with the UV detector operating at 280 nm; at this wavelength the sensitivity of this method for MMPRP is comparable to that for the other thiopurines discussed above.

The applicability of this gradient system for the high-pressure LC analysis of tissue extracts is illustrated in Fig. 4 with a chromatogram obtained from the metabolic study of ^{35}S-MP in *Adenocarcinoma 755*. The upper panel presents the profile of radioactivity found in the column effluent (1-min fractions), while the lower panel is the recording of the UV absorbance (254 nm) of the column effluent.

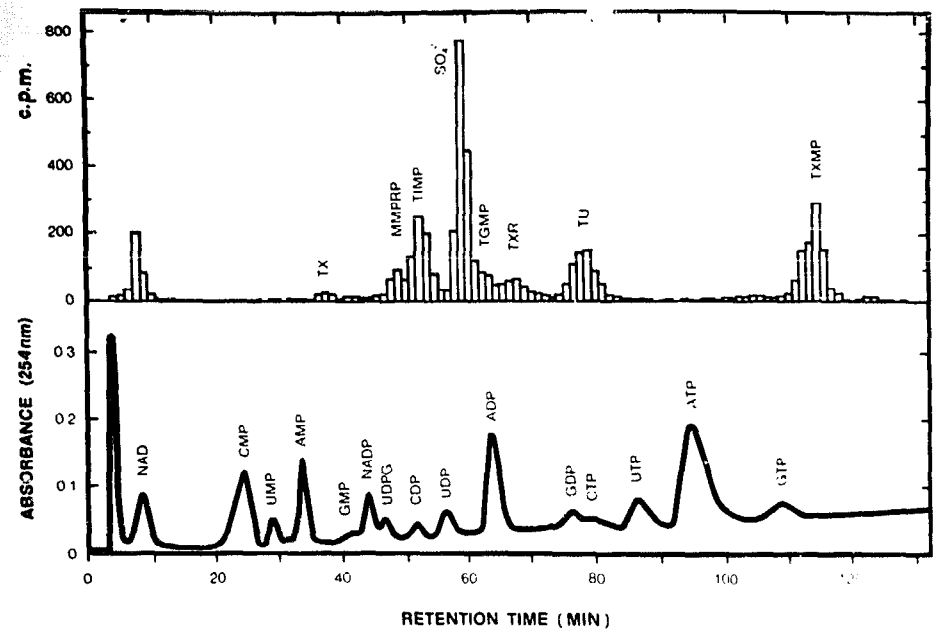

Fig. 4. High-pressure LC of acid-soluble extract from [^{35}S]MP-treated *Adenocarcinoma 755*. Tumors were excised from mice 2 h after an i.p. injection of [^{35}S]MP (25 mg/kg body weight) rapidly frozen in liquid nitrogen and extracted with perchloric acid. The neutralized acid-soluble extract of the tumors (5) was concentrated (to 1/4 the original weight of the frozen tumors) prior to chromatography. Upper panel: profile of radioactivity found in the column effluent (1-min fractions); lower panel: recording of the UV absorbance (254 nm) of the column effluent. Starting volume, 50 ml; flow-rates, 24 and 12 ml/h; eluents, 0.03 and 4.0 *M* ammonium acetate, both at pH 4.7; oven temperature, 70°; gradient delay, 15 min; sample, 15 µl (4430 d.p.m.) of neutralized, concentrated acid extract of [^{35}S]MP-treated *Adenocarcinoma 755*; UV range (254 nm), 0.32 a.u.

Where known, the identities of the peaks are included in the figure. The resolution of the major acid-soluble metabolites of [^{35}S]MP achieved by high-pressure LC approaches that obtained with CALDWELL's procedure using DEAE-Sephadex. The principal limitation of this gradient system is the poor resolution obtained with the SO_4^{2-}/TGMP/TXR triad. However, pretreatment of tissue extracts with guanylate kinase plus nucleoside diphosphokinase, to convert TGMP to TGTP ("enzymatic peak-shift")[16,18], may offer a satisfactory solution to this problem. Finally, the 254-nm tracing in the lower panel of Fig. 4 represents almost exclusively the normal nucleotide constituents of the tumor cells and illustrates the resolution obtained with these compounds under the stated operating conditions. The large peak of CMP is due to the addition of this compound as an internal standard during acid extraction of the tumors[13].

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ABBREVIATIONS

The following abbreviations have been used:
AMP, ADP, ATP = 5′-mono-, -di-, and -triphosphates of adenosine
β-ME = β-mercaptoethanol
CMP, CDP, CTP = 5′-mono-, -di-, and -triphosphates of cytidine
DEAE-Sephadex = diethylaminoethyl derivative of Sephadex
GMP, GDP, GTP = 5′-mono-, -di-, and -triphosphates of guanosine
MMP = 6-methylmercaptopurine
MMPR = 6-methylmercaptopurine riboside
MMPRP = 5′-monophosphate of 6-methylmercaptopurine riboside
MP = 6-mercaptopurine
MPR = 6-mercaptopurine riboside
MTU = 6-methylthiouric acid (6-methylthio-2,8-dihydroxypurine)
NAD, NADP = oxidized forms of nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate
8-OH-MP = 8-hydroxy-6-mercaptopurine
TEA = triethylamine
TG = 6-thioguanine
TGR = 6-thioguanosine
TGMP, TGDP, TGTP = 5′-mono-, -di-, and -triphosphates of 6-thioguanosine
TIMP = 5′-monophosphate of 6-thioinosine
TU = 6-thiouric acid
TX = 6-thioxanthine
TXMP = 5′-monophosphate of 6-thioxanthosine
TXR = 6-thioxanthosine
UMP, UDP, UTP = 5′-mono-, -di-, and -triphosphates of uridine
UDPG = uridine diphosphoglucose.

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