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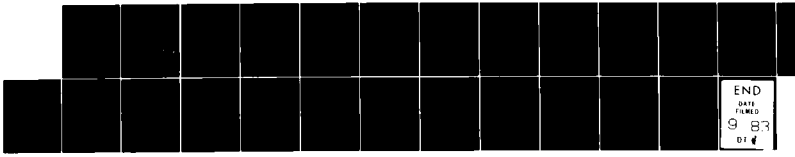

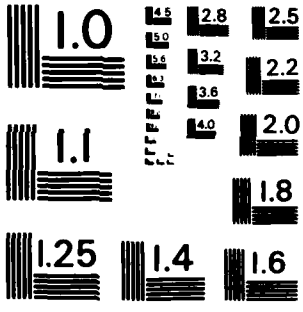

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PREPARATION OF HI-6, HGG-12 AND TOXOGONIN

ANNUAL REPORT NO. 1

M. A. Geigel and M. M. Broussard

March 1983

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1-(4-aminocarboxypyridinio)methoxymethyl-2-hydroxyiminomethyl) pyridinium dichloride (HI-6)

1,3-bis(4-hydroxyiminomethylpyridinium) dimethyl ether dichloride (Toxogonin)

Synthesis
Purification

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Published procedures for synthesis of HI-6 result in a difficult-to handle intermediate, and a product contaminated with symmetrical impurities. Because of these contaminant, purification requires repeated recrystallizations and leads to substantial material losses. We have discovered two methods that result in acceptable quality HI-6. Neither of these methods is optimized, however, and overall yields are low. We currently have 1150 g of 85% pure HI-6, prepared according to Good Manufacturing Practices (GMP).

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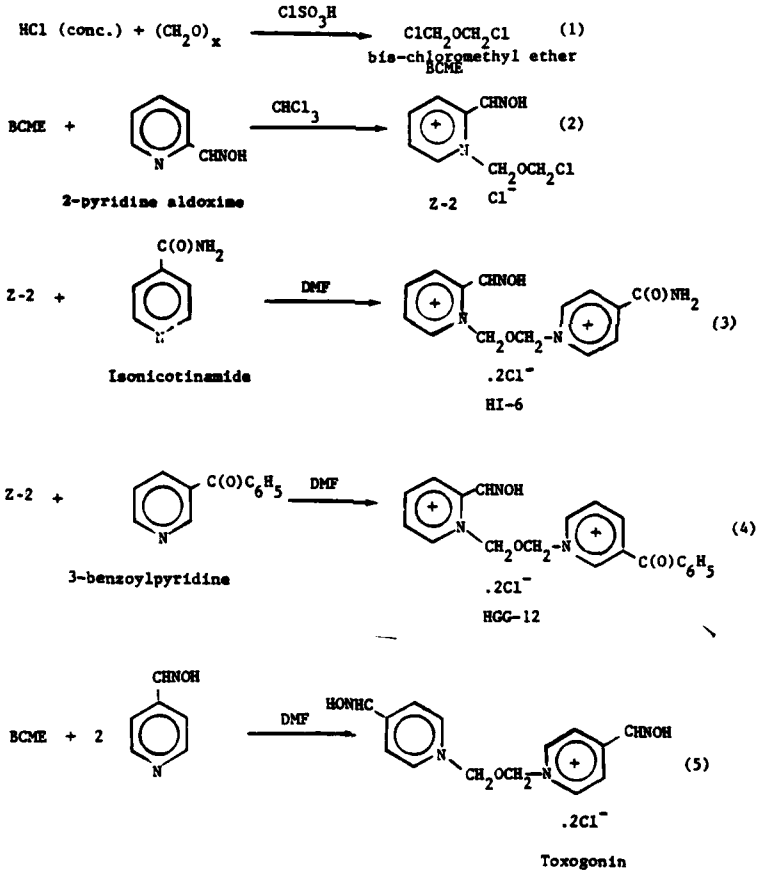
SUMMARY

Published procedures for synthesis of HI-6 result in a difficult-to-handle intermediate, and a product contaminated with symmetrical impurities. Because of these contaminants, purification requires repeated recrystallizations and leads to substantial material losses. We have discovered two methods that result in acceptable quality HI-6. Neither of these methods is optimized, however, and overall yields are low. We currently have 1150 g of 85% pure HI-6, prepared according to Good Manufacturing Practices (GMP).

The preparation of Toxogonin is much easier than that of HI-6 because the former is symmetrical. We have 680 g of 99% pure and 400 g of 94% pure Toxogonin prepared according to GMP.

INTRODUCTION

The objective of this study is to prepare 1 kg each of 1-(4-aminocarboxypyridinio)methoxymethyl-2-(hydroxyiminomethyl)pyridinium dichloride (HI-6), 1-(3-benzoylpyridinio)methoxymethyl-2-(hydroxyiminomethyl)pyridinium dichloride (HGG-12), and 1,3-bis(4-hydroxyiminomethylpyridinium) dimethyl ether dichloride (Toxogonin) in greater than 99% purity according to Good Manufacturing Practices (GMP). These materials are being prepared by the general schemes shown below:



SYNTHESIS

Bis-Chloromethyl Ether (BCME)

Containment. BCME is prepared[1] and reacted in a sealed glassware system ultimately vented through a series of caustic liquid traps. The system was designed with valves, stopcocks, and pumps to allow all manipulations of BCME without opening the system. This design conforms with the Occupational Safety and Health Administration (OSHA) definition of a closed system.

The glassware system is contained within a 1/2-inch, clear, polyvinyl chloride (PVC) glovebox that measures 5 x 3 x 4 feet and constitutes an isolated system as defined by OSHA. We considered many construction materials, and chose PVC because it offered the best combination of chemical resistance to acids, bases, and organic solvents; strength; visibility; non-permeability to BCME; and cost.

The glove box interior is maintained at a slightly lower pressure than the outside work area to further minimize the possibility of operator exposure to BCME. The glove box atmosphere is scrubbed with base before it is exhausted. Because of the pressure differential and the potential for implosion, a removable frame of 2-inch angle aluminum provides additional support for the glovebox.

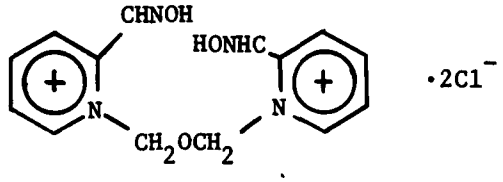
Under normal operating conditions, a blower provides the pressure differential, and a continuous stream of air is allowed to enter the glovebox through a 2-inch ball valve. A small concentration of ammonia is maintained in the atmosphere. In the event of BCME contamination of the glove box atmosphere, large amounts of ammonia will be admitted to the box to destroy the BCME immediately. The glove box will then be flushed clean of any residual BCME by completely opening the blower valve to the scrubber and a matching air inlet valve. The glove box and its accessories were designed and built by SRI.

Use of Crude BCME. We determined in initial experiments that crude, undried BCME may be used. No significant impurities attributable to the quality of BCME have been observed. The ability to use crude BCME is

important because it eliminates the need for distillation, which complicates the handling of BCME and greatly increases the hazard associated with the process, particularly at large scale-up.

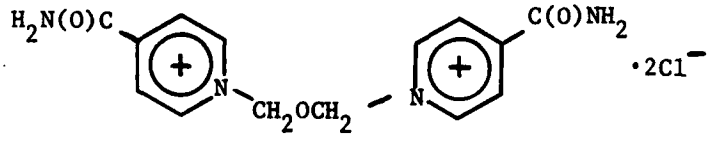
Exploratory Experiments

Hagedorn's conditions[2-4] for preparation of HI-6 yielded Z-2 as a sticky intermediate, that could neither be transferred nor filtered and washed. The resulting HI-6 was contaminated with the symmetrical analogs bis-aldoxime--1,3-bis(2-hydroxyiminomethylpyridinium) dimethyl ether dichloride (I)--and bis-isonicotinamide--1,3-bis-(4-aminocarboxypyridinium) dimethyl ether dichloride (II)--as shown below.



I

Bis-2-Aldoxime



II

Bis-Isonicotinamide

Because these impurities are structurally similar to the target compound, it is difficult to separate them. Consequently, purification of HI-6 becomes a major problem and results in substantial product losses.

We conducted exploratory runs (> 30-g scale) to determine suitable conditions for preparation of HI-6. The results of the most significant runs are summarized in Table 1. Although bis-aldoxime and bis-isonicotinamide were observed in all experiments, we discovered two methods for preparing acceptable quality HI-6.

The first method involves adding excess BCME to pyridine-2-aldoxime. The use of excess BCME minimizes the formation of bis-2-aldoxime, and appropriate washing of intermediate Z-2 minimizes the formation of the bis-isonicotinamide that forms from unreacted BCME. (Compare Runs 28, 29, and 31.) In Run 31, 40% HI-6 was obtained; the product consisted of HI-6, bis-2-aldoxime, and bis-isonicotinamide in a ratio of 10:1:1.

The second method involves the slow addition of pyridine-2-aldoxime to BCME. This is the reverse of the addition method described in the literature.[2] (Compare Runs 29 and 33.) To promote complete reaction of BCME (Step 1) so that none remains to form bis-isonicotinamide, we studied the use of an excess of pyridine-2-aldoxime (Runs 41-43). Although some bis-2-aldoxime forms because it is favored by the use of excess pyridine-2-aldoxime, this impurity is easier to remove by recrystallization than bis-isonicotinamide. As expected, the product from these runs contained less bis-isonicotinamide than products from previous runs.

Bis-isonicotinamide is formed from unreacted BCME adhering to or occluded in the crystals of intermediate Z-2. To minimize formation of bis-isonicotinamide, we tried repeated and rigorous washing of Z-2 (Runs 37 and 40) and vacuum drying overnight (Run 38) to remove unreacted BCME; however, the products of these three runs still contained 2-6% bis-isonicotinamide. Protic solvents, which react with BCME, also react with Z-2. When we washed with methanol in Run 40, a low yield was observed.

We also considered recrystallization as a means of removing unreacted

TABLE 1. PREPARATION OF HI-6, $CHCl_3$/DMF METHOD

Run	BCME	pyridine-2-aldoxime	isonicotinamide	Step 1: BCME + pyridine-2-aldoxime → Z-2[a] $CHCl_3$ (ml)	Time (hr)	Temp (°C)	Mode of Addition[b]	Comments	Crude Yield (%)	Composition of Crude HI-6 (%) HI-6	Bis-2-aldoxime	Bis-isonicotinamide	Yield HI-6[c] (%)
21	0.05	0.05	0.05	125	1	45	Lit	d	78	9	19	33	7
18	0.05	0.05	0.05	125	2	45	Lit	d	73	15	26	6	11
29	0.05	0.05	0.05	125	4	45	Lit	Hagedorn conditions	20	25	27	34	12
28	0.07	0.05	0.05	125	4	45	Lit		17	63	22	6	11
31	0.1	0.05	0.05	125	4	45	Lit		86	45	4	5	39
33	0.05	0.05	0.05	175	4	45	Reverse over 1/2 hr		18	87	8	12	16
37	0.05	0.05	0.05	75	4	45	Lit	Z-2 washed with DMF	13	57	14	6	7
38	0.05	0.05	0.05	125	4	45	Lit	Z-2 vacuum dried	44	39	8	6	17
40	0.05	0.05	0.025	75	4	45	Lit	Z-2 washed with MeOH and acetone	10	24	43	2	2
41	0.05	0.07	0.05	225	4	45	Reverse over 2 hr		15	54	6	3	8
42	0.05	0.07	0.05	225	6	60	Reverse over 4 hr		31	81	1	5	25
43	0.05	0.1	0.05	325	4	45	Reverse over 2 hr		43	55	3	6	24
36	0.1	0.05	0.05	125	4	45	Lit		57	34	9	3	19
32	0.05	0.05	0.05	75	4	45	Lit		96	34	22	6	33
39	0.05	0.05	0.05	25 ml $CHCl_3$ + 30 ml DMF	2	45	Reverse over 2 hr	Isonicotinamide added to BCME; intermediate reacted with oxime	57	2	0	60	

[a] Z-2 was filtered, washed with 3 x 50 ml chloroform, and reacted with isonicotinamide in 250 ml DMF for 2 hr at 45°C.

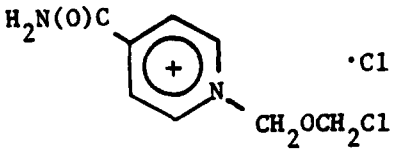
[b] "Lit" signifies BCME was added to oxime over 10 min; "reverse" signifies oxime added to BCME.

[c] Crude yield x % yield HI-6.

[d] Filtered and washed Z-2 was added to clean reaction flask.

BCME. Although recrystallization of Z-2 is reported to cause its decomposition,[5] we tried test-tube-scale purifications without success. For example, 1 mg of Z-2 was not soluble in 1 ml of dimethyl formamide, tetrahydrofuran, dioxane, acetonitrile, or nitromethane; Z-2 was soluble in dimethyl sulfoxide, but it could not be reprecipitated by acetone dilution.

Another way to address the problem of residual BCME caused by the sluggish reaction of pyridine-2-aldoxime with BCME is to reverse the order of substitution and add isonicotinamide slowly to excess BCME (Run 39). We hypothesized that the rapid reaction of isonicotinamide with BCME would mean that no isonicotinamide would be available to react with the mono-isonicotinamide-substituted BCME (Y-2) whose structure is shown below.



Y-2

This hypothesis would be correct if the reaction of Y-2 with isonicotinamide is slower than the reaction of BCME with isonicotinamide, or if Y-2 precipitated before it can react further. Any residual BCME left in Y-2 after filtering and washing would yield, in Step 2, bis-2-aldoxime, which is easier to remove. Our results did not, however, bear out the hypothesis. Bis-isonicotinamide was the major product isolated from Run 29.

To determine whether isonicotinamide is displacing the pyridine-2-aldoxime portion of HI-6 to give bis-isonicotinamide, we heated HI-6 and isonicotinamide in dimethylformamide (DMF) for 2 hr at 45°C. No formation of bis-isonicotinamide was observed.

A comparison of Runs 29 and 32 shows the effect of reactant concentration. As expected, higher concentrations result in higher yields. Unfortunately, for the preferable reverse addition method, the amount of chloroform used is governed by the solubility of pyridine-2-

aldoxime, especially when it is added at room temperature through a dropping funnel. In larger scale and process work, we could add more concentrated, hot solutions through metering pumps. A high yield in Step 1 means a small amount of residual BCME; consequently, a smaller amount of bis-isonicotinamide was observed in Run 32. The substantial amount of bis-2-aldoxime obtained in that run is common when a stoichiometric amount of BCME is added to the oxime.

Runs 31 and 36 illustrate the reproducibility of our technique. The most difficult part of the method to reproduce is the washing of Z-2.

One-Solvent-System

Because of the difficulty in handling Z-2, we explored an alternative to the two step-two solvent ($CHCl_3$/DMF) procedure: We prepared Z-2 in DMF solvent and, without isolating the Z-2, we added isonicotinamide. This route offers the great advantages of simplicity and minimal need for manipulation of BCME-containing streams. However, in all cases in which we used this system (see Table 2), significant quantities of bis-isonicotinamide were formed. Although we believe it is possible to adjust stoichiometry, time, and temperature to optimize HI-6 formation and minimize by-product formation, we did not explore this route further because the project goal is to prepare material and not to develop a process.

Table 2. PREPARATION OF HI-6, ALL DMF METHOD

Run	Conditions[a] DMF in Step 1 (mL)	Time (hr)	Yield (%)	Crude HI-6 (%) HI-6	Bis-2-Aldoxime	Bis-isonicotinamide
12	25	0.75	67	22	0	25
24	100	1.5	56	23	0	55
22	200	1.5	50	8	0	67
19	200	3	17	3	0	90
25	100	3	56	21	1	39
27	100	3[b]	67	29	5	37

[a]0.05 mole BCME was added to 0.05 mole pyridine 2-aldoxime in the specified amount of DMF, and heated at 45°C for the specified time; 0.05 mole isonicotinamide in 50 mL DMF was then added to the reaction mixture, which was heated at 45°C for the specified time.

[b]Reaction mixture was heated at 60°C; Run 26, heated at 80°C, turned black.

PRODUCTION

HI-6

For production of HI-6, we chose Method 1, the slow addition of excess BCME to pyridine-2-aldoxime. We chose this method over Method 2 for two reasons: (1) BCME is relatively easy to synthesize, whereas pyridine-2-aldoxime is expensive; (2) In our closed system the addition of solids is difficult. The feasible scale of reaction using Method 2 is then governed by the limited solubility of pyridine-2-aldoxime in the chloroform solvent.

The current inventory of HI-6 is 1154 g of 85% pure material as shown in Table 3.

Table 3

INVENTORY OF HI-6

Source (Pilot Plant Run No.)	Quantity (g)	Average Purity (%)
P3, 8, 10, 11,	326	87
P14, 15	290	80
P16	141	85
P17	178	82
P19	82	85[a]
P20	64	85[a]
P21	73	85[a]
Total	1153	

a) Analysis by nuclear magnetic resonance (nmr); other analyses are by high pressure liquid chromatoraphy (hplc).

Given the results of initial, small-scale recrystallizations, we expect to recover ∿900 g of 99% HI-6 (∿75% recovery).

Notable in Table 3 are the decreased yields in Runs P19-21. In these runs we found larger amounts of the sticky material always formed with the intermediate Z-2. In Run P18, the sticky material predominated, and the isolated final product was mostly bis-2-aldoxime. In Run P19, the crystalline Z-2 was separated from the sticky material, and the two fractions were treated separately in the usual manner. The former fraction yielded HI-6 of acceptable quality, but in decreased yield; the latter fraction yielded mostly bis-aldoxime. We postulate that the sticky material is predominantly bis-aldoxime that contains some Z-2 and some unreacted BCME. Its formation may be a complex function of the rate of addition of BCME, the form and rate of precipitation of Z-2, stirring rate, temperature of pyridine-2-aldoxime solution when BCME is added, and other, subtle, unidentified factors.

The conditions being used are by no means optimized, and the aspects of the reaction scheme that present problems include:

- Handling of Z-2. This intermediate has varied from a sticky material that adheres to the stirring rod to a very hard solid that is difficult to remove from the flask.
- Washing of Z-2. The physical form of Z-2 makes washing difficult, yet incomplete washing results in the difficult-to-remove bis-isonicotinamide impurity.
- Product color. The crude product ranges from light to dark gray, and is sometimes green.
- Sensitivity to operator. Production Run 7, conducted by a different operator, yielded poor results.
- Variation in ratio of HI-6 to impurities.
- Corrosion of equipment and resulting malfunction caused by HCl and ammonia fumes inside the glove box. Ammonia vapors are used to react with any escaped BCME.
- Substantial recrystallization losses (∿50%) through attempts to remove small amounts (<5%) of impurities and traces of color.
- Poor and Variable yields.

Toxogonin

Because Toxogonin is a symmetrical molecule, its preparation does not present the problems of by-product formation encountered with HI-6. Our present inventory includes 680 g of 99% Toxogonin. We expect that recrystallization of an additional 400 g of 94% Toxogonin will yield ~320 g of 99% material. A final recrystallization will be performed to combine all fractions into a homogeneous lot.

HGG-12

Preparation of HGG-12 will begin in the next quarter.

PURIFICATION

Because even the best synthesis scheme tried to date produces HI-6 contaminated with some symmetrical analogs, a suitable purification method was required. Although recrystallization is the preferred technique, we also investigated the possibility of using ion exchange, adsorptive, and high-pressure liquid chromatography (hplc).

Recrystallization

Because crude, vacuum-dried material inevitably contains residual DMF solvent, the first purification step consists of a precipitation. Because BCME is hydrolyzed by water, precipitation of HI-6 from a water solution has the advantage of assuring that no BCME will be present in the product.

We tested a number of systems for recrystallization of precipitated HI-6:

Water/acetone

Water/methanol

Water/ethanol

Water/iso-propanol

Methanol

Ethanol, 95%

methanol/ether

Pure water was not tested because of the high solubility of HI-6 in that solvent. Conversely, because HI-6 is insoluble in most organic solvents, they cannot be used.

Several solvent systems removed bis-aldoxime, but bis-isonicotinamide proved more difficult to remove. Many solute concentrations and ratios of water to organic solvent were tried; some of these are summarized in Table 4.

For production, recrystallizations are carried out slowly and carefully in 75% ethanol/water, the optimal solvent system. Because HI-6

TABLE 4. RECRYSTALLIZATION OF HI-6

Sample	Condition		Yield	Percent Organics[a]		
	HI-6	Solvent		HI-6	Bis-2-aldoxime	Bis-isonicotinamide
Crude Run 31				45	4	5
31-1	15 g crude 31	450 ml 89% MeOH/ether[b]	8	96	0	4
31-2	31-1	MeOH	-	97	0	3
Crude Run 32				34	22	6
32-1	17 g crude 32	650 ml 61% MeOH/ether[b]	22	81	15	5
32-2	4 g 32-1	300 ml MeOH	31	94	1	5
32-2-1	0.2 g 32-2	0.4 g 50% MeOH/water	80	95	0	5
32-2-2	0.2 g 32-2	0.6 g 50% MeOH/water	70	96	0	4
32-2-3	0.2 g 32-2	0.8 g 50% i-PrOH/water	80	96	0	4
32-2-4	0.2 g 32-2	30 g 95% EtOH/water	-	94	0	5
32-2-2-1	0.08 g 32-2-2	1 ml 70% EtOH/water	62	99	0	0.5
Crude 36				34	9	3
36-1	1 g crude 36	140 ml Acetone/MeOH/water 5/1/1[b]	10	69	25	3
36-1-1	0.1 g 36-1	0.5 g 50% MeOH/water	33	77	19	0.5
36-1-2	0.1 g 36-1	0.9 g 66% MeOH/water	10	97	2	1
36-1-3	0.1 g 36-1	1 g iPrOH/MeOH/water 2/1/1	33	84	12	1
36-1-4	0.1 g 36-1	1 g 50% MeOH/water/3% HCl	19	74	26	0.5
36-1-5	0.1 g 36-1	1 g iPrOH/MeOH/water 1/1/2	60	80	19	1
Crude 37				57	14	6
37-1	2 g crude 37	150 ml 66% MeOH/ether[b]	50	83	9	7
37-2 crop 1	1 g 37-1	23 ml 87% MeOH/water	20	98	1	1
37-2 crop 2	-	-	10	93	1	6
Crude 42				81	1	5
42-1	6 g crude 42	250 ml 80% MeOH/water[b]	59	96	0	4
42-2	3 g 42-1	40 ml 75% EtOH/water	58	99	0	1
42-3	1.7 g 42-2	20 ml 75% EtOH/water	50	99	0	0.5

[a]Certain samples have water of hydration.

[b]Precipitation, not true recrystallization.

decomposes in solution, recrystallizations are carried out at pH 3-4, the range of maximum stability. Charcoal treatment is used to remove color.

Ion Exchange Chromatography

HI-6, bis-2-aldoxime, and bis-isonicotinamide differ most prominently in the number of oxime and amide functionalities they contain. We decided to capitalize on this feature to separate the compounds by ion exchange chromatography. When the oxime is in its anion form, bis-aldoxime, HI-6, and bis-isonicotinamide are neutral, a monochloride salt, and a dichloride salt, respectively.

A cation exchange resin may be effective in separating HI-6 from its symmetrical analogs, especially in removing bis-isonicotinamide. The results of our attempts are summarized in Table 5. Percentage recovery of HI-6 has not yet been determined, and some decomposition of HI-6 is anticipated at higher pH levels.

Although recrystallization is the simplest way to purify the required material, if chromatography is required, ion exchange would be preferred for several reasons: First, the amount of resin required is only 3-5 times the amount of material to be purified (as opposed to 50-100 times for silica gel or alumina). Second, the resin can be regenerated. Third, ion exchange chromatography is faster than adsorptive chromatography.

Adsorptive Chromatography

HI-6 may be separated from bis-isonicotinamide by silica gel thin layer chromatography using 15% aqueous sodium chloride/methanol at a ratio of 5:1. With this solvent system and silica dry column chromatography, we obtained HI-6 free of organic impurities, but contaminated with sodium chloride from the elution solvent. Suitable silica gel or alumina conditions free of sodium chloride have not been found. Conditions for hplc require the use of an ion-pairing reagent, which would also contaminate the eluted HI-6.

TABLE 5. PURIFICATION OF HI-6 BY ION EXCHANGE[a] CHROMATOGRAPHY

Sample	Conditions[b]	Percent HI-6	Bis-2-aldoxime	Bis-isonicotinamide
38-1	35 mg HI-6	80	12	6
38-6-3	35 mg HI-6; 0.5 g resin	77	18	1
38-6-4		89	7	0.5
38-6-5		76	20	0.7
38-6-6		61	31	1.4
38-7-2	1 g HI-6 3 g resin	96	3	0.5
38-7-2		98	1	0.1
38-7-4		89	7	2
38-7-5		88	6	3
38-8-1	35 mg HI-6 brought to pH 8; 0.5 g resin	5	62	19
38-8-2		74	13	0
38-8-3		36	8	0

[a] Bio-Rex 70 cation exchange resin.

[b] Elute with water.

Analytical Methods

Table 6 outlines various hplc conditions reported for the analysis of HI-6. Separation of all important components is possible, as illustrated in Figure 1. Propiophenone is used as the internal standard. The use of a new column and modified conditions have solved initial problems of unacceptable resolution, retention time shifts, and nonreproducibility.

TABLE 6. CONDITIONS FOR HPLC ANALYSIS OF HI-6 AND TOXOGONIN

	Benschop Paper[a]	Contract DAMD17-79-C-9154 SRI Project 8906[b]	Contract DAMD17-82-C-2073 SRI Project 4168
Instrument	Waters Associate Model 6000A	Waters Associate Model 6000A	Spectra Physics 3500B
Detector	Tracov 970 A; 304 nM	Perkin Elmer LC-75; 300 nM	Chromatronix 225; 254 nM.
Column	RP-18; 5 microns	Waters RCM-CN; 10 microns	Waters μ-Bondapak CN; 10 microns
Mobile phase	30% methanol/70% 0.1 M sodium acetate buffer (pH 4.80) with 4.6 mM sodium n-octanesulfonate and 0.3 mM dimethyl octylamine	30% Methanol/70% 0.1 M KH_2PO_4 (pH 7.1)	10% Acetonitrile/ 90% 0.025 M sodium pentanesulfonate
Flowrate	0.2 ml/min	1.5 ml/min	1.0 ml/min
Integrated	LDC-304-50 computing integrator	Hewlett-Packard 3390A	Hewlett-Packard 3380A

[a] Hendrick P. Benschop, et al., Journal of Chromatography, 225, 107 (1981).

[b] Report No. 404.

Table 4. CONDITIONS FOR HPLC ANALYSIS OF HI-6 AND TOXOGONIN

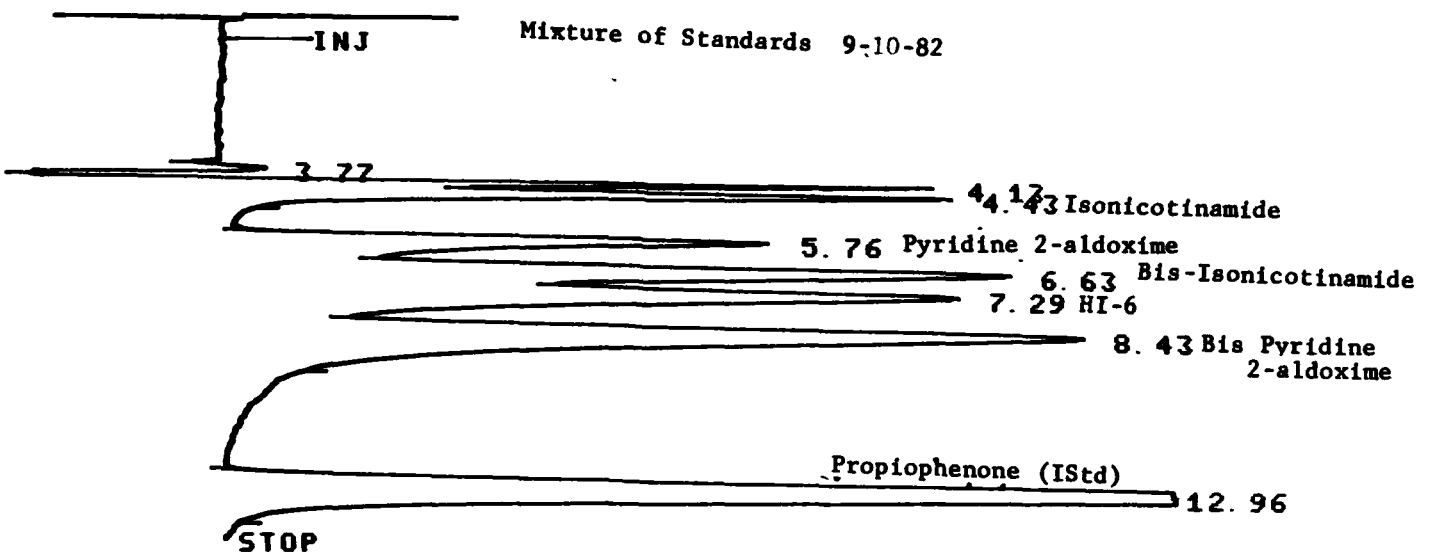


Figure 1. HPLC SEPARATION OF IMPORTANT SYSTEM COMPONENTS

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1. S. Buc, Org. Syn., Coll. Vol. IV, 101 (1963).

2. I. Hagedorn (to Merck), "Bis-quaternary Pyridinium Salts," U.S. Patent 3,852,294 (December 3, 1974).

3. I. Hagedorn (to Merck), "Bis-quaternary Pyridinium Salts," U.S. Patent 3,773,775 (November 20, 1973).

4. I. Hagedorn (to Merck), "Bis-quaternary Pyridium-2-Aldoxime and their Preparation," German Patent 26 16 481 (November 3, 1977).

5. K. Schoene, "Darstellung and Untersuchung neuer Acetylcholinesterase-Reaktivatoren," Dissertation, University of Freiburg (1967).

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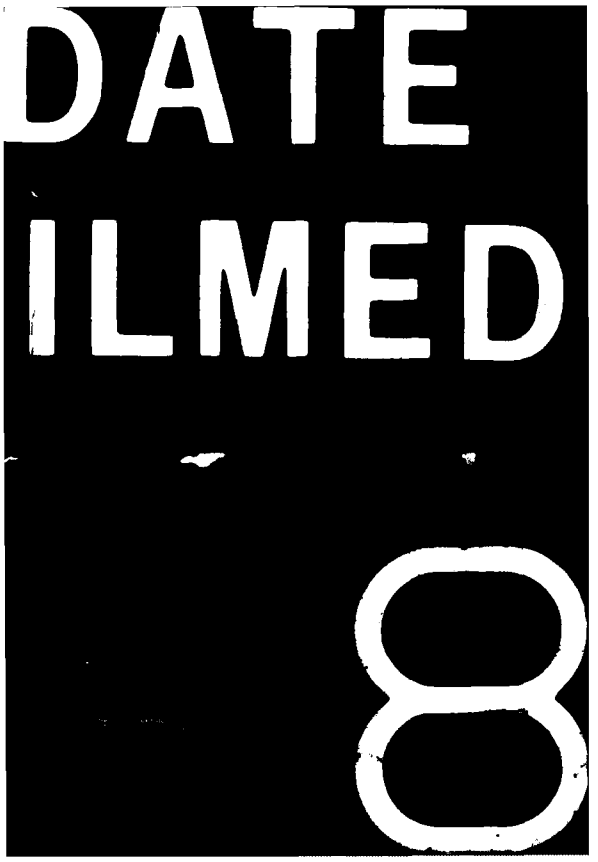
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