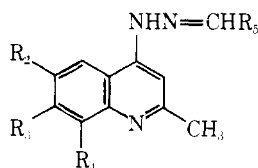
Table IV. Free-Wilson Substituent Constants

Substituent	No. of occurrences	Computed FW substituent constants
R_2 = CH_3O	10	1.39
R_2 = H	34	−0.41
R_3 = C_2H_5O	10	0.64
R_3 = H	34	−0.19
R_4 = CH_3O	12	−0.02
R_4 = H	32	−0.009
R_5 = 2-$CH_3OC_6H_4$	4	−0.07
R_5 = 3-$CH_3OC_6H_4$	4	0.90
R_5 = 4-$CH_3OC_6H_4$	4	1.80
R_5 = 2,5-$(CH_3O)_2C_6H_3$	4	−0.90
R_5 = 2,3-$(CH_3O)_2C_6H_3$	4	−0.93
R_5 = 3,4-$(CH_3O)_2C_6H_3$	2	0.73
R_5 = 2,4-$(CH_3O)_2C_6H_3$	4	−0.48
R_5 = 3,4,5-$(CH_3O)_3C_6H_2$	4	1.24
R_5 = 2,4,5-$(CH_3O)_3C_6H_2$	3	−0.33
R_5 = 4-Methoxynaphthyl	3	−0.12
R_5 = 9-Anthracenyl	4	−1.03
R_5 = 9-Ethyl-6-carbazolyl	4	−0.70

lution is supported by the consistency of the findings and not by statistical criteria such as the *F* ratio, since including a number of inactive compounds with the same index of activity distorts the measure of residual variation in the study.

Using this method of analysis it can be concluded that in quinolinehydrazones of the type



(i) R_2 = CH_3O is more favorable for activity than R_2 = H; (ii) R_3 = C_2H_5O is more favorable for activity than R_3 = H; (iii) in the R_4 position H and CH_3O groups are comparable; (iv) of the R_5 groups studied, the preferred substituents are R_5 = 3-$CH_3OC_6H_4$, 4-$CH_3OC_6H_4$, 3,4-$(CH_3O)_2C_6H_3$, or 3,4,5-$(CH_3O)_3C_6H_2$.

From the structures of three compounds (Table III, no. 5, 6, 21) that provided total protection at the doses used, we are again persuaded to conclude that CH_3O at R_2, C_2H_5O at R_3, and 3-, 4-, or 3,4,5-methoxylation of the phenyl ring at R_5 are activity-enhancing substituent groups. The two other compounds tested that allowed 100% survival (Table I, no. 17, 37) were excluded from the matrix because the R_5 substituents (C_6H_5 and 3-FC_6H_4) were poorly represented. Nevertheless, they again support the conclusion that in this series R_3 is optimally C_2H_5O. The data also suggest that the substitution pattern R_1 = CH_3, R_2 = CH_3O, R_3 = C_2H_5O, and R_5 = 3-$CH_3OC_6H_4$, 4-$CH_3OC_6H_4$, 3,4-$(CH_3O)_2C_6H_3$, or 3,4,5-$(CH_3O)_3C_6H_2$ would result in more potent compounds on a dosage basis.

We conclude that in the study described the Free-Wilson approach has been of significant value in displaying the data and accommodating both active and inactive compounds to allow potentially useful qualitative conclusions to be drawn.

Acknowledgment. We wish to thank Dr. J. W. Wilson for his helpful suggestions and interest during the course of this work.

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Preparation and Anticoagulant Activity of Trimethylsilyl Heparin in Carbowax

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Trimethylsilyl heparin, when administered intraduodenally or intragastrically to rats, did not increase intestinal absorption and, consequently, the clotting times were not influenced. However, suspension of sodium heparin in Carbowax 200 prolonged the whole blood clotting time at a dose of 50 mg/kg when given intraduodenally or intragastrically to rats.

Heparin is a mucopolysaccharide of high molecular weight (17,000–20,000) normally isolated from mammalian tissue. It is used as an anticoagulant for blood as well as in many clinical situations such as in thrombophlebitis, phlebothrombosis, arterial occlusions, and as prophylaxis against thrombosis after trauma to blood vessels, etc.[1] It is usually administered by subcutaneous, intramuscular, or intravenous injection since it is inactive or only slightly active (at very high doses) when given orally. An orally active heparin would have many applications particularly for prophylactic use.

A number of attempts have been made in the past to make a suitable heparin derivative which can be absorbed through the intestinal walls but these approaches have met with only limited success. Koh and Bharucha have claimed preparations of a number of stable, orally active heparinoid

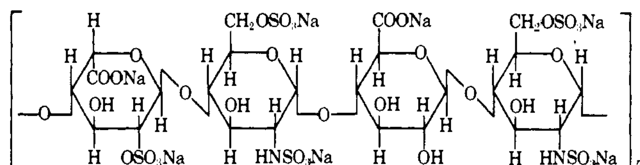

Figure 1.

complexes,[2-5] but these proved to be ineffective in clinical testing. Recently, another report[6] has suggested an oral activity for a λ-heparin.

Since a lipophylic substance is considered to be more readily absorbed from the gut and since the highly charged nature of heparin (Figure 1)[7,8] was likely to be part of the reason for lack of oral activity, trimethylsilyl derivatives of heparin were prepared and tested for *in vivo* activity in rats and rabbits.

Results and Discussion

Since heparin is a highly charged polyanionic polysaccharide (Figure 1), it was thought that by reduction of this charge through derivatization the lipophilicity of heparin would be increased which in turn might increase its absorption in the gut. Because the trimethylsilyl derivatives of several polysaccharides have been reported in the literature, preparation of trimethylsilyl derivatives of heparin was investigated. Prior to this work Speier[9] had reported a trimethylsilyl derivative of heparin, which when injected into a dog prevented blood from clotting for a substantially longer period of time than an equal amount of nonsilylated sodium salt of heparin. Repeated attempts in our laboratories to duplicate these results met with total failure. Silicon analysis on the product indicated very little evidence of any silylation, as compared to complete silylation of heparin claimed by Speier.[9]

Our initial efforts were directed toward using the standard silylation procedure[10-12] using chlorotrimethylsilane and hexamethyldisilazane in the presence of pyridine as solvent. As mentioned earlier, these conditions used successfully by Speier[9] failed to give satisfactory results in our hands. One of the primary reasons for the observed low, if any, degree of silylation using the above procedure may be because heparin was in suspension and not in solution. Based on the reported success in dissolving starch in formamide,[13] sodium heparin was dissolved in formamide by heating for 1 hr at 65°. This solution was treated with chlorotrimethylsilane, hexamethyldisilazane, and pyridine under the usual or varying silylation conditions. Silicon analysis on the product indicated that partial silylation of heparin had occurred. Using this method, the observed degrees of substitution which resulted ranged between 1.34 and 1.54 (heparin A and B, respectively, Table I), whereas the theoretical value, based on silylation of all the free hydroxyl groups, is 5, and, based on silylation at all the other positions, is 12. One distinct disadvantage of this procedure is the contamination of the product with ammonium chloride and/or pyridine hydrochloride, both by-products of the reaction. This assumption was confirmed by the unusually high nitrogen content and positive halogen test on the product. Attempted purification of these partially silylated heparin samples by dialysis was unsuccessful. Therefore, we investigated other procedures used for preparing trimethylsilyl derivatives. One such procedure involves the use of hexamethyldisilazane as the only solvent as well as silylating reagent.[10] A formamide solution of sodium heparin was treated with a large excess of hexamethyldisilazane. Silicon analysis on the product indicated that almost complete silylation (based on the hydroxyl positions the observed degree of substitution was 4 out of 5) of heparin had taken place (Table I). In one instance a higher degree of substitution (7.16) was obtained but it could not be reproduced. Its nitrogen content was only slightly higher than the theoretical value. This could possibly be due to the formation of ammonium salts of some of the carboxyl groups present in heparin, since free ammonia gas was liberated during the reaction.

Table I. Silicon, Nitrogen, and Sulfur Analyses (%) and Degrees of Silylation of Sodium Heparin

Sample[a]	Residue as Na_2SO_4, %	N	S	Si	Deg of substn
Sodium heparin	40.00	2.14	12.11		
A		9.74		2.80	1.34
B		9.02		3.20	1.54
C	17.50	2.62	6.12	11.50	7.16
D	20.70	2.85	4.10	7.25	3.92
E	19.10	3.39		7.25	3.92

[a]Samples A and B have been prepared by using method A. Samples C, D, and E have been prepared by using method B. The high degree of silylation of sample C could not be reproduced.

Table II. Comparison of the Activities of Heparin, Silyl Heparin, and the Hydrolyzed Product (Desilylated) from Silyl Heparin

Activity, units/mg		
Heparin	Silyl heparin	Desilylated silyl heparin
133	104	125

The above trimethylsilyl derivative of heparin was found to be soluble in dimethylformamide (DMF), dimethyl sulfoxide (DMSO), and Carbowax 200.

The silylated heparin samples were characterized by silicon and nitrogen analyses and infrared spectroscopy. The infrared spectra of these derivatives, as Nujol mulls, had characteristic strong peaks at 845 and 1250 cm^{-1}. The degree of substitution given in Table I was calculated from silicon analysis.

The nuclear magnetic resonance spectrum of the trimethylsilyl derivative of heparin had peaks characteristic of the trimethylsilyl groups.

Stability of this derivative in water was studied polarimetrically. The results showed complete hydrolysis of the trimethylsilyl groups in about 2 hr. The product on recovery showed no peaks, characteristic of C–Si vibrations. The activity of this hydrolyzed product was determined and compared with that of heparin. The values were 125 and 133 units/mg (Table II), respectively. The small deviation could be explained by the fact that the activities of the two samples were not determined at the same time under identical conditions. The products gave ir peaks identical with that of heparin.

Gel electrophoresis[14] of unreacted heparin and silyl heparin gave identical single spots.

These evidences establish the fact that heparin has not been decomposed to any smaller fragments during the reaction for silylation.

Biological Results. Silylized heparin preparations A–E were not absorbed by small intestine any more effectively than sodium heparin when given to rats intraduodenally

Table III. WBCT ($\bar{x}$ ± S.E. of 6) of Rats Receiving Heparin or Silylized Heparin A–E (see Materials) id at 50 mg/kg in 1 ml of Carbowax 200

Treatment	Time (min) after dose 0	30	60	90
Carbowax 200 control	235 ± 32	250 ± 15	253 ± 19	230 ± 18
Sodium heparin	195 ± 33	767 ± 179[a]	342 ± 41	205 ± 30
Heparin A	208 ± 6	250 ± 32	198 ± 36	173 ± 44
Heparin B[b]	150	170	632	415
Heparin C	172 ± 26	148 ± 23	188 ± 33	187 ± 28
Heparin D	197 ± 26	222 ± 59	250 ± 54	223 ± 20
Heparin E	198 ± 4	237 ± 26	250 ± 58	240 ± 52

[a]Statistically significant, $p < 0.05$. [b]Data on one animal only.

(id) at 50 mg/kg in 1 ml of Carbowax 200. Whole blood clotting time was significantly affected only in the animal receiving sodium heparin (Table III). A suggestion of activity was seen with heparin B at 1 hr but due to insufficient supply of compound it was tested in only one rat. Preliminary experiments in rats showed that intragastric administration of sodium heparin was effective at 50 mg/kg when given with 1 ml of Carbowax 200 per 250 g of body weight.

Sodium heparin was absorbed in the small intestine more readily when suspended in Carbowax than in water, corn oil, or Tween 80. When heparin was given to rats (id) at 25 mg/kg with 1 ml of Carbowax 200 per 250 g of body weight, WBCT was delayed (Table IV). Higher doses of heparin in the same volume of Carbowax 200 produced a greater and longer lasting effect. The anticoagulant effect was lost when less (0.5 ml/250 g) Carbowax 200 was used. Carbowax 400 and 1000 were tested but were no more effective than Carbowax 200. In Tween 80 heparin activity was seen only at 100 mg/kg id when given 1 ml per 250 g of body weight. In water or corn oil no activity was produced at 100 mg/kg.

When heparin was given to rats ig, an anticoagulant effect was seen but this effect was short lived, occurred only at one time interval tested and this interval varied between animals from 1 to 2 hr after dosing. This suggested heparin was not absorbed in the stomach. In order to test this hypothesis, heparin was injected into the stomach of a rat which had its gut tied off at the pyloric sphincter. WBCT in these animals showed inconsistent results but when injected distal to the pyloric sphincter, a consistent delay of WBCT was observed. These results suggest that heparin can be absorbed from the duodenum consistently, but absorption from the stomach is not predictable.

We examined the silylized preparations for anticoagulant activity *in vitro* and found heparins A, B, and C were approximately the same as sodium heparin; heparins D and E were less effective (Table V). These results do not correlate with the degree of substitution and no explanation could be found for these results.

Table IV. Number of Rats (of 6) Showing Anticoagulation (WBCT >2× Control) Following Sodium Heparin Administration id in Carbowax 200 (1 ml per 250 g of Body Weight)

mg/kg	Time (min) after heparin 0	15	30	60	90	120
25	0	2	2	0	0	0
50	0	6	5	3	0	0
100	0	6	6	4	2	0

Table V. *In Vitro* Coagulation Time (Recalcification Time in sec) of Rat Blood in the Presence of Heparin or Silylized Heparin Preparations

	Concn, μg/ml[a] 100	50	10	5	1	Saline
Sodium heparin	>	>	>	450	280	270
Heparin A	>	>	>	600	335	315
Heparin C	>	>	>	690	340	330
Heparin D	>	>	475	320	315	315
Heparin E	>	670	325	330	340	350

[a]> = greater than 600 sec.

Experimental Section

The infrared spectra were run as Nujol mulls on a Beckman IR8 spectrophotometer. The nuclear magnetic resonance spectra were obtained on a Varian HA-100 spectrometer using DMSO-d_6 as solvent and no internal standard. Elemental analysis was performed by Midwest Microlab Inc., Indianapolis, Ind.

Preparation of Trimethylsilyl Derivative of Heparin. Method A. Using Chlorotrimethylsilane and Hexamethyldisilazane in the Presence of Pyridine and Formamide. In a three-necked round-buttomed flask equipped with a condenser and mechanical stirrer, sodium heparin (2 g) was dissolved in formamide (20 ml) by heating at 65° with stirring for about 1 hr. The solution was cooled to room temperature and dry pyridine (50 ml) added to it, whereby sodium heparin precipitated as a fine suspension. A mixture of chlorotrimethylsilane (20 ml) and hexamethyldisilazane (40 ml) was added to the above suspension dropwise with vigorous stirring. The reaction mixture was allowed to stir at room temperature for 24 hr and poured into benzene (350 ml). The solvents were removed by decantation; the residual solid was washed several times with absolute ethanol followed by ether. Filtration and drying at 100° under vacuum (0.1 mm) for 12 hr afforded 2.2 g of the trimethylsilyl derivative as a white granular powder. Its infrared spectrum as Nujol mull had strong peaks at 845 and 1250 cm^{-1}, characteristic of the C–Si bond. Silicon and nitrogen analyses and the degrees of silylation are given in Table I.

Method B. Using Hexamethyldisilazane in the Presence of Formamide. Sodium heparin (20 g) and formamide (200 ml) were heated at 60° for 1 hr in a 2-l. three-necked round-bottomed flask equipped with a mechanical stirrer and a reflux condenser. The solution was allowed to cool to room temperature, hexamethyldisilazane (500 ml) was added, and the reaction mixture was heated at 70° for 2 hr with vigorous stirring. Liberation of ammonia gas was noticed during the course of the reaction. The reaction mixture was cooled to room temperature and poured into anhydrous acetone (500 ml), whereby the trimethylsilyl derivative of heparin separated out. Solvents were removed by decantation and the white residue was washed repeatedly with anhydrous acetone by stirring and decantation. The product (23 g) was isolated by filtration and dried at 100° under vacuum for 12 hr. Its infrared spectrum as Nujol mull had strong peaks at 845 and 1250 cm^{-1}, characteristic of C–Si bond. Silicon and nitrogen analyses and the degree of silylation are given in Table I.

Stability Study of Water Solution of the Silylated Heparin by Polarimeter. A 1% solution of the silylated heparin in water was made. The solution was quickly transferred in a polarimeter tube which was placed immediately in the polarimeter; the change in rotation was continuously recorded on chart paper for 2 hr, after which time the rotation became constant signifying the complete hydrolysis of the silyl heparin. The change in rotation during the first hour was more rapid than during the second hour.

Electrophoresis of Heparin and Silyl Heparin. Electrophoresis was done with a standard gel electrophoresis instrument. The gel was made from a 0.9% solution of agarose in a barbital buffer solution of pH 8.6 on microscope slides. A 2-μl sample of heparin

or silyl heparin (0.1%) in water was applied and electrophoresis was run at 550 V, 18 mA, for 70 min. The spots were developed with Toluidine Blue indicator. Identical spots were obtained at about 3 cm from the points of application on the slide.

WBCT of Rat Blood. Cyclopal was given intravenously (iv) to anesthesia. An abdominal incision was made, the inferior vena cava was exposed, and 0.5 ml of blood was drawn with a 1-ml Stylex (Pharmaseal Labs., Glendale, Calif.) syringe and 26 G × 0.5 in. needle. At 20 sec from venipuncture 0.3 ml of blood was placed in a Fibrocup and the clotting time determined with a Fibrometer (B.B.L., Baltimore 18, Md).

WBCT of Rabbit Blood. Cyclopal was given iv to anesthesia and an abdominal incision made to expose the inferior vena cava. Blood samples (1.1 ml) were drawn with a 1-ml syringe and 26 G × 0.5 in. needle. Aliquots (0.5 ml) of blood were placed in disposable 12 × 75 mm culture tubes approximately 20 sec from venipuncture. The tubes were placed in a water bath (37°) and tilted every 30 sec to see if the blood was fluid. WBCT was recorded when blood was no longer fluid and would not flow up the side of the tube.

Anticoagulant Activity *in Vitro.* Heparin was dissolved in and serially diluted with saline. Aliquots (0.05 ml) were placed in disposable 10 × 75 mm culture tubes containing 0.1 ml of 0.25 *M* $CaCl_2$ in saline. Blood (9 ml) was drawn from rats into syringes containing 3.8% citrate (1 ml) and mixed. Aliquots of blood (0.9 ml) were placed in culture tubes containing the heparin and $CaCl_2$ solutions and placed on a Lab-Tek mixer (Ames Lab-Tek, Westmont, Ill.) and the time to clotting was recorded as recalcification time. Comparisons between sodium heparin and silylized heparin were made by their ability to prolong the recalcification time. Comparisons between sodium heparin and silylized heparin were made by their ability to prolong the recalcification time.

Heparin Administration. Compounds were given ig to fasted (>16 hr) animals or id by injecting through the stomach wall through the pyloric sphincter into the duodenum using a syringe and 19 G × 1 in. needle.

Suspension or Solution of Compounds. Heparin preparations were suspended in Carbowax, Tween 80, or mineral oil by sonication (<10 sec) to give a homogeneous suspension or dissolved in saline prior to mixing with a vehicle. Carbowax 1000 was warmed to approximately 40° in order to suspend the heparin.

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Chemotherapeutic Nitroheterocycles. 18.† 2-(5-Nitro-2-imidazolylmethylene)-1-indanones, -1-tetralones, and -acetophenones Substituted by Aminoalkoxy Groups

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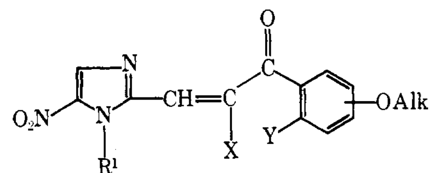
2-(5-Nitro-2-imidazolylmethylene)-1-indanones, -1-tetralones, and -acetophenones substituted by aminoalkoxy groups and related compounds (**41–69**, Table II) were synthesized and their antimicrobial activities were evaluated (Table III). Some of these compounds (*e.g.*, **47**, **52**, and **59**) surprisingly exhibited a broad antibacterial spectrum including *Proteus* species and *Pseudomonas aeruginosa.* Extraordinary antitrichomonal activities could also be observed *in vitro* (MIC of compound **59**, 0.0004 μg/ml) and six of the title compounds (**48**, **49**, **52**, **58**, **64**, **66**) displayed *in vivo* activity in mice against *Trichomonas vaginalis* comparable to that of metronidazole (**70**).

In two previous papers of this series[2,3] 5-nitro-2-imidazolylmethylene derivatives of alkoxy-1-indanones (**1**), alkoxy-1-tetralones (**2**), and alkoxyacetophenones (**3**) were shown to possess considerable activity against *Trichomonas vaginalis* (*T. vaginalis*) *in vitro;* however, in general their *in vivo* efficacy in mice (subcutaneous) was disappointing. The rather poor solubility of the compounds in inorganic and organic solvents may lead to an extremely low absorption rate from the gastrointestinal tract which would explain the discrepancy between *in vitro* and *in vivo* activity.

In order to enhance the solubility we intended to introduce substituted amino groups into the alkoxy residue (OAlk in formulas **1–3**) of the compounds as similar modifications within the series of 5-alkoxy-2-(5-nitro-2-furfurylidene)-1-indanones led to low MIC values against *T. vaginalis.*[4]

This communication is concerned with the synthesis and the results of the microbiological screening of 2-(5-nitro-2-imidazolylmethylene)-1-indanones, -1-tetralones,



1, –X, Y– = $-CH_2-$
2, –X, Y– = $-C_2H_4-$
3, –X, Y– = –H,H–

and -acetophenones substituted by aminoalkoxy groups and some related structures (physical and chemical data are given in Table II; Table III shows the microbiological results).

Chemistry. The methods used for the synthesis of the compounds listed in Table II are given in Scheme I.

The starting materials, 4-, 5-, 6-, and 7-hydroxy-1-indanone, 6-hydroxy-1-tetralone, and 4′-hydroxyacetophenone (compounds **4–9**), were purchasable or known from the literature. Alkylation with 2-bromoethanol led to the 2-hydroxyethoxy derivatives (method A, **10–13**) which could be

† For part 17 of this series, see ref 1.