is apparent. Whether treatment with 5 × 0.1% solution or 3 × 1% solution is concerned, a coefficient of about 0.17 is obtained. This means that in each of the experiments evaluated, the activity of the ethanolamine salts is about 50% higher than that of the corresponding free acids (the antilogarithm of 0.17 is 1.47).

If the general term "activity" has been used so far, this—strictly speaking—is not quite correct. Defined precisely, one ought to state that the rate-determining step in the mechanism of action of this experiment is controlled exclusively by the parameter π.

However, in this relatively simple animal model only the penetration into the *stratum corneum* can be thought of as the rate-determining step.

We have no satisfactory mechanistic explanation at the moment as to why the activity of a compound can be increased by about 50% if it is used not in its free form but as the ethanolamine salt. A possible explanation would be that there is an influence by the ethanolamine on environmental factors in the area of infection or on the motility of the compounds in the aqueous epidermal layer.

Acknowledgment. We are indebted to Professor von Wasielewski and D. M. Vanderbeke for stimulating discussions of these results.

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Nitrofurans with High Renal Excretion

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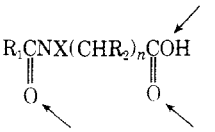
During the last 20 years a considerable number of nitrofurans with high antibacterial activity have been synthesized, but only a few of these are found to be excreted in the urine in an antibacterially active form.[1,2] Nitrofurantoin is one example of a nitrofuran compound with a high urinary excretion. This may partly be ascribed to its tubular excretion.[3,4] Other urinary tract antibacterial agents of sulfonamide[5] and penicillin[6] type are also excreted tubularly. To obtain new urinary tract antibacterial nitrofurans, therefore, it would be advantageous to synthesize nitrofuran compounds which are likely to be excreted in the tubules.

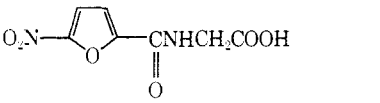
Despopoulos has systematized various compounds having a renal tubular excretion similar to that of 4-aminohippurate.[7] All these compounds are acids. The renal tubular excretion transport of these organic acids is described as a substrate-specific process involving an interaction between a transported anion and a receptor molecule in the renal cell. Despopoulos gave the general structural requirements (structure 1) for compounds with tubular excretion. The two oxygens of the carboxyl group and the oxygen of the carbonyl group were assumed to be essential for the three-point contact with the receptor.



1. X = H, alkyl; n = 1–5

On the basis of Despopoulos's general formula 1, we embarked on the synthesis of the nitrofurans **2–4** containing those groups considered essential for tubular excretion (Chart I and Table I). Since the acids **3** and **4** were synthesized *via* their esters, the latter compounds were included for comparison.

Chart I



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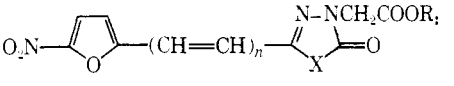

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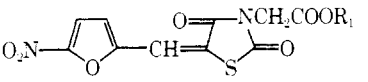
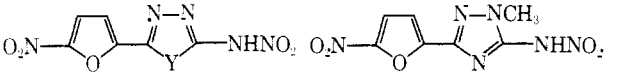
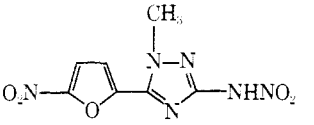


4



5 **6**



7

X = O, S, NCH_3; Y = NH, NCH_3, S; R_1 = H, ethyl

In addition, a series of nitrofurans with a nitroamino group (**5–7**) was synthesized since we had found earlier that 4-methyl-3-nitroamino-5-(5-nitro-2-furyl)-1,2,4-triazole (**5b**) is excreted tubularly (Chart I and Table I).[8] The antibacterial activity *in vitro* and the urinary excretion of the new nitrofurans **2–7** were examined.

Synthesis and Chemical Data. The nitrofurans **3** and **4** were synthesized from the corresponding nitrofuran compounds in which the heterocyclic ring had a NH adjacent to the C=O group. The sodium salt was prepared and then allowed to react with ethyl chloroacetate or ethyl bromoacetate. The ester group was then hydrolyzed in concentrated H_2SO_4–formic acid. The nitrofurans **5–7** with a nitroamino group were obtained by nitration of the corresponding amino compounds.[8]

The pK_a values of the acidic nitrofurans were determined by potentiometric titration and are reported in Table I. All the nitrofurans **2–7** were stronger acids than nitrofurantoin and exist at physiological pH (7.2) mainly as anions.

Biological Data. I. Antibacterial Activity *in Vitro.* The minimal inhibitory concentrations (MIC values) of the nitrofurans **2–7** against *Staphylococcus aureus*, *β-haemolytic streptococcus*, and *Escherichia coli* were determined by the twofold serial dilution technique.[9] Furthermore, some of the nitrofurans were tested using the same

Table I. Chemical and Biological Data

Compd	n	X	Y	R_1	Method	Yield, %	Mp, °C	pK_a	Antibacterial act. *in vitro*, MIC, μg/ml				Total urinary excretion in dogs,[d] % of the dose given			
									St-a[b]	β-Str	E-c	Tri-v	No. of dogs	Without probenicid	No. of dogs	With probenicid	Tubular excretion
2						41	148–151.5		>200	>200	>200		4	29 ± 9	3	5, 9, 16	
3a	0	NCH_3		C_2H_5	A	32	127–129		12.5	>200	50	1.5[c]	4	66 ± 16	4	59 ± 11	+
3b	0	NCH_3		H	C	65	203.5–206	3.6	100	>200	100	0.02[c]	5	42 ± 14	3	13, 17, 28	+
3c	0	S		C_2H_5	A	71	130–132		25	>200	200	4[c]	2	5, 5			
3d	0	S		H	C	81	160–163	3.5	>200	>200	>200	0.02[c]	6	62 ± 8	3	3, 12, 14	+
3e	0	O		C_2H_5	A	73	115–118		12.5	>200	50	1.5[c]	5	34 ± 9	2	2, 10	+
3f	0	O		H	C	62	153–158	3.5	>200	>200	>200	0	2	37, 62	4	18 ± 11	+
3g	1	O		C_2H_5	A	76	153–157		12.5	>200	>200	3[c]	4	<1–3			
3h	1	O		H	C	61	211–214	3.5	50	200	200		3	35, 16, 9	3	2, 3, 4	+
4a	0			C_2H_5	A		177.5–179.5		3.2	>200	200	4[c]	2	0, 0			
4b	0			H	C	37	214–216 dec		50	200	>200		2	1, 1			
5a			NH^a		D	75	168 dec	3.4	>200	>200	100		5	25 ± 9	2	26, 26	
5b			NCH_3		D	59	160 dec	4.2	50	200	50	0.06[c]	7	29 ± 10	2	3, 6	+
5c			S		D	62	185 dec		25	100	12.5		2	31, 48	3	<1, 1, 2	+
6					D	54	149 dec	3.6	100	>200	50		3	12, 12, 23	3	7, 16, 30	
7					D	82	132 dec	3.5	25	>200	200		6	11 ± 3	3	11, 13, 18	
8		Nitrofurantoin						7.2	12.5	50	6.3	0.1[c]	26	19 ± 8	11	7 ± 5	+

[a]Formula **5** is one of three tautomeric forms. [b]St-a = *Staph. aureus*, β-Str = *haem. β-strept.*, E-c = *E. coli*, Tri-v = *Trichomonas vaginalis*. [c]MIC value of furazolidone/MIC value of nitrofuran. [d]Individual values or mean value ± standard deviation.

technique against *Trichomonas vaginalis*. As the reproducibility of the MIC values in this test was unsatisfactory, the activity is expressed in relation to that of furazolidone in the same test (MIC value of furazolidone/MIC value of nitrofuran). The MIC values of nitrofurantoin were included for comparison (Table I).

The antibacterial activity of the new nitrofurans was low. The esters were more active than the acids. Against *Trichomonas* the esters **3c**, **3e**, and **4a** show very high activity, but this was lost when the ester was hydrolyzed to the corresponding acid.

Our results are in accordance with the earlier observations[10-13] that the antibacterial activity, especially against gram-negative bacteria, is strikingly decreased when nitrofurans are substituted with acidic groups. This may be due to an inability of the nitrofurans to penetrate bacteria as anions. Nitrofurantoin is a weaker acid than the nitrofurans **2–7** and is only partly dissociated at physiological pH (7.2).

II. Urinary Excretion in Dogs. The peroral absorption and the urinary excretion of the nitrofurans **2–7** were tested in dogs. A single dose of 5 mg/kg was given orally and the total urinary excretion was measured for 0–6 hr, using a polarographic assay technique.[14] This method measures the total amount of nitrofuran compounds excreted in the urine as a sum of the parent nitrofuran compound and all possible metabolites containing an intact nitrofuran group.

For studying tubular excretion probenicid (100 mg/kg) was administered together with the nitrofuran compound. Probenicid is known to block the acid transport system in the renal tubular cell.[15] The excretion data are reported in Table I and nitrofurantoin is used as a reference substance.

Five (**2**, **3b**,**d**,**f**,h) of six nitrofurans with the essential groups for tubular excretion showed a very high excretion, 20–62%. Further, their excretion was blocked by probenicid, indicating a tubular excretion. Two of the esters, **3a** and **3e**, also showed a high excretion, 66 and 34%, respectively. However, no unchanged ester compound could be demonstrated by tlc in dog urine. Probably, **3a** and **3e** were rapidly hydrolyzed in the body. However, probenicid did not block the excretion of **3a**. The reason for this is unknown.

4a has the essential groups for tubular excretion but is not excreted in the urine. This fact may be due to poor absorption of **4a** from the intestinal tract. Neither are other nitrofurfurylidenethiazolones excreted in the urine.†

The nitrofurans **5–7** were excreted over a longer period than the nitrofurans **2–4**. In some cases the excretion still continued after 6 hr. The excretion values of **5–7** are therefore minimum values and might have been a little higher if the collection time had been longer. Three of the five nitroamino compounds, **5a–c**, showed a higher urinary excretion than nitrofurantoin. The excretion of **5b,c** was blocked by probenicid. However, the excretion of **5a**, **6**, and **7** was not blocked by probenicid.

The blocking of the excretion of **5b,c** by probenicid indicates a tubular secretion of these compounds but further studies are required to confirm this.

Conclusions

Despopoulos's general formula 1 for compounds with tubular excretion has been found to be valid for nitrofurans even when essential structural elements shown in 1 are incorporated in a heterocyclic ring. Certain nitrofurans with

†E. Åkerblom, personal communication.

a nitroamino group are also rapidly excreted, probably by the tubular acid transport system.

It has been possible to design nitrofurans with a high urinary excretion which is probably due to tubular secretion. Their high urinary excretion, which is superior to that of nitrofurantoin, indicates good peroral absorption and slow metabolic inactivation. Excretion by the tubular acid transport system requires that the nitrofurans are acids. However, acidic groups decrease the antibacterial activity, especially against gram-negative bacteria. Although nitrofurantoin is also an acid, it is too weak to be completely dissociated at physiological pH, which may explain its high antibacterial activity. Whether it is possible to design nitrofurans with tubular excretion and a higher antibacterial activity than nitrofurantoin, or whether the optimum has been attained with nitrofurantoin, has yet to be established.

Experimental Section

The structures of all compounds were assigned on the basis of compatible ir, nmr, and mass spectra and satisfactory analyses. The melting points are uncorrected. All compounds were analyzed for C, H, N, and S and the analytical results were within ±0.4% of the calculated values.

Chemistry. Method A. Synthesis of 3a,c and 4a. Starting material for the synthesis of **3a** was 4-methyl-5-(5-nitro-2-furyl)-1,2,4-triazolin-3-one,[8] for **3c** 5-(5-nitro-2-furyl)-1,3,4-thiadiazolin-2-one, and for **4a** 5-(5-nitro-2-furfurylidene)thiazolidine-2,4-dione.[17] The sodium salts of the starting materials were prepared by adding 0.05 mol of sodium methylate dissolved in 30 ml of DMF to a mixture of 0.05 mol of the starting material and 40 ml of DMF. To the sodium salt 5.3 ml (0.05 mol) of ethyl chloroacetate was added and the mixture was heated at 90° for 2 hr. DMF was then evaporated *in vacuo* and the residue was treated with water. A crude product precipitated. **3c** and **4a** were recrystallized from ethanol. Repeated recrystallizations were required to obtain **4a** in pure form. **3a** was purified from the starting material by dissolving in acetone. The starting material was then removed by filtration. The acetone solution was evaporated and the residue recrystallized from water to give pure **3a**.

Method B. Synthesis of 3e and 3g. The starting material for synthesis of **3e** was 5-(5-nitro-2-furyl)-1,3,4-oxadiazolin-2-one[18] and for **3g** 5-(5-nitro-2-furyl)vinyl-1,3,4-oxadiazolin-2-one.[19] The sodium salts of the starting materials were prepared by dissolving 0.025 mol of the starting material in 40 ml of DMF and then adding in portions 1.2 g (0.025 mmol) of NaH (dispersed in oil) over a period of 45 min at 10–15°. To the sodium salt, 4.2 g (0.025 mol) of ethyl bromoacetate was added dropwise. The reaction solution was stirred for 3 hr at room temperature and then poured onto 200 g of ice. The product precipitated and was filtered and recrystallized from ethanol.

Method C. Synthesis of 3b,d,f,h and 4b. **3a,c,e,g** and **4a** were hydrolyzed by adding 0.007 mol of each to a mixture of 0.4 ml of concentrated H_2SO_4 and 18 ml of HCOOH and heating the mixture at 70–80° for 24 hr. The solution was then cooled and **3d,h** and **4b** precipitated. **3b,f** were obtained by evaporating the HCOOH *in vacuo*. **3b,d** and **4b** were recrystallized from water, **3f** from ethyl acetate–CCl_4, and **3h** from acetonitrile.

Method D. Synthesis of 5a–c, 6, and 7. For synthesis of **5a** the starting material was 3-amino-5-(5-nitro-2-furyl)-1,2,4-triazole, for **5b** 3-amino-4-methyl-5-(5-nitro-2-furyl)-1,2,4-triazole, for **5c** 2-amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole, for **6** 3-amino-2-methyl-5-(5-nitro-2-furyl)-1,2,4-triazole, and for **7** 3-amino-1-methyl-5-(5-nitro-2-furyl)-1,2,4-triazole.[8] The starting material (0.01 mol) was added in small portions to a mixture of 0.7 ml (0.01 mol) of concentrated HNO_3 and 6 ml of concentrated H_2SO_4 at 0° over a period of 20 min. The mixture was stirred for another 60 min at 0° and then poured onto ice. The product was collected by filtration and **5a** and **7** were recrystallized from methanol, **5b** from ethanol, and **6** from ethanol–DMF. **5c** was recrystallized from methanol–DMF and then reprecipitated in water.

***N*-(5-Nitro-2-furoyl)glycine (2).** Glycine (34 mmol) was dissolved in 75 ml of water and pH was adjusted to 8.5. A solution of 6 g (34 mmol) of 5-nitro-2-furoyl chloride in 14 ml of dioxane was added dropwise to the above solution at room temperature over a period of 35 min. The pH of the solution was kept at 8.5 by continuous addition of diluted NaOH. The reaction was completed by stirring for 1 hr. The reaction solution was acidified with concentrated HCl to pH 0.5 and extracted with ethyl acetate. The ethyl acetate phase was collected, dried, and evaporated to give 6.1 g of crude product. This was recrystallized from ethyl acetate: yield, 3.0 g of **2**.

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Potential Anticancer Agents. 10. Synthesis of *N*-[4-[[(2,4-Diamino-7-pteridyl)methyl]-N^{10}-methylamino]benzoyl]glutamic Acid

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N-[4-[[(2,4-Diamino-6-pteridyl)methyl]-N^{10}-methylamino]benzoyl]glutamic acid (I, Methotrexate), synthesized in 1949 at the American Cyanamid Co.,[1] proved to be one of the powerful inhibitors of dihydrofolate reductase[2] and a very effective anticancer agent.[3] In order to explore this structural area, we decided to prepare one of its closely related analogs, the 7-isomer II.

The original procedure for the synthesis of I[1] involved the condensation in aqueous medium (pH 3–3.5) of tetraaminopyrimidine (III) with the sodium salt of *N*-[4-(*N*-methylamino)benzoyl]glutamic acid and 1,2-dibromopropionaldehyde. In alternate routes of synthesis, the use

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