(1.93 g, 8.00 mmol) in AcOH (75 mL). After 15 min at ~25 °C, the solution was poured into Et_2O (500 mL). The solid that formed was collected and washed with Et_2O followed by H_2O to give pure **11d**.

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Studies on Antimicrobial Agents. 1. Synthesis and Relation between the Antimicrobial Activities and Certain Physicochemical Properties of Some *N'*-(Pyridinioacetyl) Fatty Acid Hydrazides[1]

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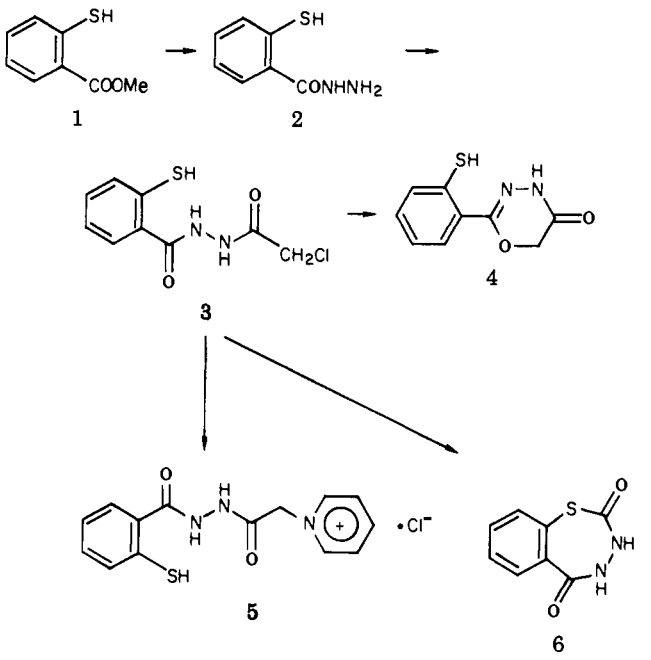
A new series of *N'*-(pyridinioacetyl)alkanoic and -benzoic acid hydrazides, as chloride salts, and some cyclic analogues produced by ring closure have been synthesized and tested in a search for more effective germicides. Physicochemical parameters, such as surface tension, critical micelle concentration, and thermodynamic activity (Ferguson values), were also determined. *Staphylococcus aureus* and *Streptococcus pyogenes* were the most susceptible of the organisms tested. *N'*-(Pyridinioacetyl)hexadecanoic acid hydrazide exhibited the highest toxicity to *Staph. aureus* and fungi. The mean surface tension of the equitoxic solutions is 59.8 ± 0.3 dyn/cm for bacteria and 51.65 ± 0.1 dyn/cm for fungi. *N'*-(Pyridinioacetyl)octadecanoic acid hydrazide and *N'*-(pyridinioacetyl)-9-octadecenoic acid hydrazide exhibit the highest toxicity to *S. pyogenes* (1.1×10^{-6} M). The surface tension of their equitoxic solutions and their Ferguson values indicate that these compounds may act through a different mechanism.

With the disclosure by Domagk[2] of the much improved germicidal activity obtained when a large aliphatic residue was attached to the quaternary nitrogen atom, the study of this type of compound was greatly stimulated. The chemotherapeutic applications of quaternary ammonium salts have gained importance since World War II; many members of this class with a potent bactericidal and antifungal activity have found general utility in skin disinfectants, as well as in the formulation of creams, ointments, lotions, etc. Although in the past 40 years many patents have been issued in the field, few improvements on benzalkonium chloride[2] were reached.

In a further exploration of the influence of chemical structure upon the antimicrobial activity of quaternary ammonium salts, some of the compounds described in an earlier paper[3a,b] were modified by the introduction of a bishydrazide residue into the pharmacophoric group. Hence, a series of 20 new *N'*-(pyridinioacetyl)alkanoic and -benzoic acid hydrazides as chloride salts has been prepared (Table I). These compounds and their cyclic analogues (Scheme I) produced by ring closure, 5,6-dihydro-2-(mercaptophenyl)-4*H*-1,3,4-oxadiazin-5-one (**4**) and 3,4,5,6-tetrahydro-2*H*-1,4,5-benzothiadiazocine-3,6-dione (**6**), previously reported,[4] were evaluated for in vitro antimicrobial activity against several strains of bacteria and fungi. Physicochemical parameters such as surface tension, critical micelle concentration, and thermodynamic activity (Ferguson values) were determined.

Scheme I



Chemistry. The *N'*-(pyridinioacetyl)alkanoic and -benzoic acid hydrazides, **1–20**, were prepared in a conventional manner[4,5] as shown in Scheme I. When **1** was

(1) This work forms part of a thesis submitted by one of us (S.M.S.) to the University of Buenos Aires for the Degree of Doctor in Experimental Pharmacology.
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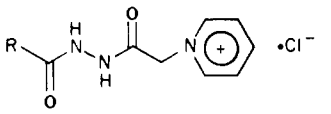
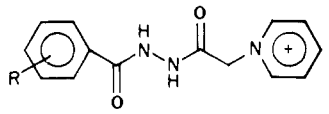
Table I. Physical Properties of N'-(Pyridinioacetyl)alkanoic and -benzoic Acid Hydrazide Chloride Salts



no.[a]	R	mp,[b] °C	yield,[c] %	γ,[d] dyn/cm, ±SD
1	C_3H_7	172-173	80.3	64.42 ± 0.01
2	C_4H_9	180-181	85.1	64.12 ± 0.01
3	C_5H_{11}	190-191	79.0	62.82 ± 0.02
4	C_7H_{15}	199-200	95.2	51.12 ± 0.01
5	C_9H_{19}	201-202	97.0	45.21 ± 0.01
6	$C_{11}H_{23}$	191-192	90.7	43.42 ± 0.02
7	$C_{13}H_{27}$	189-190	90.9	41.51 ± 0.01
8	$C_{15}H_{31}$	182-183	82.3	39.91 ± 0.02
9	$C_{17}H_{35}$	180-181	89.9	42.52 ± 0.01
10	Δ^9-*cis*-$C_{17}H_{33}$	180-181	92.78	40.50 ± 0.01



11	H	275-276	75.2	62.38 ± 0.01
12	o-OH	257-258	89.1	63.25 ± 0.01
13	o-SCH_2Phe	198-199	80.6	63.66 ± 0.01
14	o-$SCH_2CH{=}CH_2$	193-194	98.0	62.42 ± 0.02
15	o-$SOCH_2$Phe	227-228[e]	95.2	53.26 ± 0.02
16	o-SO_2CH_2Phe	240-241	82.0	51.17 ± 0.01
17	o-OCH_2Phe	224-225	82.0	65.46 ± 0.01
18	o-Cl	212-213[e]	85.0	64.99 ± 0.02
19	*m*-NO_2	256-257	80.6	61.22 ± 0.01
20	*p*-NO_2	277-278	76.5	58.17 ± 0.01
	o-SH	unstable		

[a] All were analyzed for C, H, and N, and results were within ±0.03% of theory. [b] After recrystallization from ethanol-ethyl acetate, with dec. [c] Yield of the recrystallized product in the last reaction step. [d] Surface tension of 0.1% aqueous solution at 22 ± 1 °C. [e] Hygroscopic.

treated with refluxing hydrazine hydrate, the corresponding hydrazide (**2**) was readily formed. ^1H NMR spectra shows signals characteristic of the $-NHNH_2$ group: a singlet at 9.5–10 ppm (NH) and a broad signal at 4.2–4.5 ppm for NH_2. Symmetric substitution of the hydrogen atom of the hydrazide group was carried out by treatment of hydrazide **2** with chloroacetyl chloride.

The NMR spectra of N'-(chloroacetyl)alkanoic and -benzoic acid hydrazides (**3**) show two singlets at 10.5–11.7 ppm corresponding to the hydrogens of the $-CONHNHCO-$ group. Reaction of **3** with an excess of dry pyridine leads to pyridinio salts (**5**). The NMR spectra of these salts show no displacement of the signals and, in addition, exhibit the exchangeable protons of the CH_2-N group. The N'-(pyridinioacetyl)-2-mercaptobenzhydrazide (**5**) is an unstable product which could not be isolated. The oxidation of compounds **15** and **16** was carried out before the quaternization.

In Vitro Antimicrobial Assays. Preliminary tests were made using the disk-plate method.[6] The assay compounds were dissolved in water (**1–7** and **11–20**), in 50% Me_2SO–water (**4**, **6**, and **8**), and 100% Me_2SO (**9** and **10**). Hydrazides that exhibited activity at a concentration of 1000 μg/mL were studied further to determine their minimal inhibitory concentration (MIC). The autotitration method[7] used in this work is highly correlative with the tube dilution test. Data on MIC values are given in Table II.

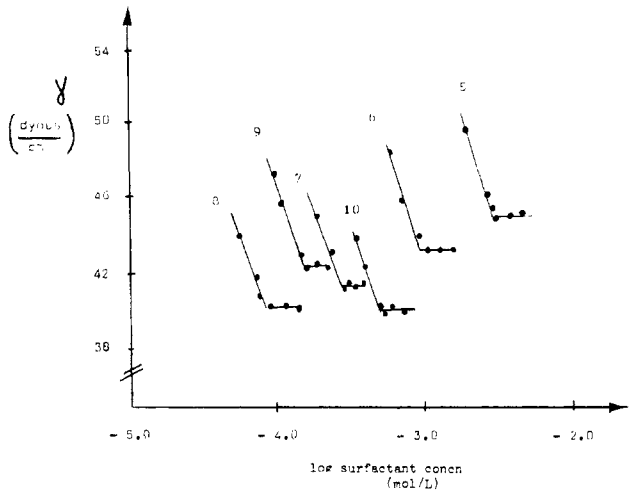


Figure 1. Graphs of surface tension (γ) against log surfactant concentration used to determine the critical micelle concentration of compounds listed in Table IV.

Physicochemical Properties. Surface tensions of the compounds synthesized were measured in aqueous solutions by the ring-detachment method (Table I). The plots of surface tension against concentration (not shown) for each surfactant agent were concave to the axes. The surface tension (Table III) of equitoxic aqueous solutions, based on MIC values of the bactericides, were read from these plots. Critical micelle concentrations (cmc) have been measured by the surface-tension method.[8] The results of the surface-tension method for each N'-(pyridinioacetyl) fatty acid hydrazide gave a graph on which the cmc was interpreted from the intercept of two straight lines (Figure 1). Below this value, the surface tension decreased linearly with increases in the log surfactant concentration. Above it, the surface tension was almost constant. The thermodynamic activity (Ferguson values) of solutions of N'-(pyridinioacetyl) fatty acid hydrazide surfactants below the cmc was determined as the ratio of equitoxic solution concentration (MIC) and the cmc (see Table IV).

Results and Discussion

Some of the new bishydrazide quaternary salts reported in this paper possess broad-spectrum in vitro antimicrobial activity against representative bacteria and fungi, as shown in Table II. The highest activity is shown against Gram-positive bacteria, such as *Staphylococcus aureus* and *Streptococcus pyogenes*, with MIC values as low as 4.5×10^{-6} and 1.1×10^{-6} M, respectively. Only compound **6** (at the concentrations used) shows activity against *Escherichia coli* and *Pseudomonas aeruginosa*, but of a low order (3.4×10^{-4} M). This is consistent with the observation that in a given series the compounds most active against Gram-positive organisms are more lipophilic than those most active against Gram-negative ones.[9,10]

The introduction of the bishydrazide group resulted in a diminution of activity (i.e., MIC for *Staph. aureus* for hexadecylpyridinium chloride is 0.002% w/v and for

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Table II. Minimal Inhibitory Concentrations (MIC, mol/L) of N'-(Pyridinioacetyl) Fatty Acid Hydrazides

organism	compound 4	5	6	7	8	9	10
Staph. aureus Smith	$>1.6 \times 10^{-3}$ [a]	3.66×10^{-4}	8.5×10^{-5}	1.96×10^{-5}	4.6×10^{-6}	$>3.4 \times 10^{-5}$	$>1.7 \times 10^{-5}$
E. coli Vogel	$>1.6 \times 10^{-3}$	$>1.5 \times 10^{-3}$	3.4×10^{-4}	$>3.9 \times 10^{-5}$ [b]	$>3.7 \times 10^{-5}$	$>6.9 \times 10^{-5}$	$>6.9 \times 10^{-5}$
K. pneumonia 39645	$>1.6 \times 10^{-3}$	$>1.5 \times 10^{-3}$	6.8×10^{-4}	$>7.9 \times 10^{-5}$	$>7.4 \times 10^{-5}$	$>6.9 \times 10^{-5}$	$>6.9 \times 10^{-5}$
P. vulgaris 9920	$>1.6 \times 10^{-3}$	$>1.5 \times 10^{-3}$	6.8×10^{-4}	$>7.9 \times 10^{-5}$	$>7.4 \times 10^{-5}$	$>6.9 \times 10^{-5}$	$>6.9 \times 10^{-5}$
P. mirabilis MGH-1	$>1.6 \times 10^{-3}$	$>1.5 \times 10^{-3}$	1.4×10^{-3}	$>7.9 \times 10^{-5}$	$>7.4 \times 10^{-5}$	$>6.9 \times 10^{-5}$	$>6.9 \times 10^{-5}$
Ps. aeruginosa MGH-2	$>1.6 \times 10^{-3}$	$>1.5 \times 10^{-3}$	3.4×10^{-4}	$>3.9 \times 10^{-5}$	$>3.7 \times 10^{-5}$	$>3.4 \times 10^{-5}$	$>3.4 \times 10^{-5}$
S. pyogenes C-203	1.6×10^{-3}	3.7×10^{-4}	4.2×10^{-5}	9.8×10^{-6}	9.1×10^{-6}	1.1×10^{-6}	1.1×10^{-6}
C. albicans 10231	$>1.6 \times 10^{-3}$	7.3×10^{-4}	1.7×10^{-4}	1.96×10^{-5}	1.8×10^{-5}	$>1.7 \times 10^{-5}$	$>1.7 \times 10^{-5}$
As. niger 16404	$>1.6 \times 10^{-3}$	1.5×10^{-3}	1.7×10^{-4}	3.9×10^{-5}	3.7×10^{-5}	$>3.4 \times 10^{-5}$	$>3.4 \times 10^{-5}$
T. mentagrophytes 9129	$>1.6 \times 10^{-3}$	1.5×10^{-3}	1.7×10^{-4}	3.9×10^{-5}	3.7×10^{-5}	$>1.7 \times 10^{-5}$	$>1.7 \times 10^{-5}$

[a] The MIC is greater than 1.6×10^{-3} M. [b] The MIC is greater than 3.9×10^{-5} M, but due to the effect of the solvent no MIC can be determined.

Table III. Surface Tension of an Equitoxic Solution of N'-(Pyridinioacetyl) Fatty Acid Hydrazides

no.	surface tensions (γ), dyn/cm, 22 °C[a] *S.a*	*S.p*	*C.a*	*A.n.*	*T.m*
5	60.2	60.2	53.0	47.5	47.5
6	60.0	62.0	54.0	54.0	54.0
7	59.5	59.5	54.5	54.0	54.5
8	59.5	57.5	52.3	47.0	47.0
9		72.0			
10		72.2			

[a] Abbreviations used: *S.a., Staph. aureus; S.p., S. pyogenes; C.a., C. albicans; A.n., A. niger; T.m., T. mentagrophytes.*

Table IV. Critical Micelle Concentrations and Thermodynamic Activities of N'-(Pyridinioacetyl) Fatty Acid Hydrazides

no.	cmc, M	thermodynamic activity,[a] cmc/MIC *S.a*	*S.p*	*C.a*	*A.n*	*T.m*
5	3.70×10^{-3}	0.098	0.098	0.197	0.394	0.394
6	8.91×10^{-4}	0.095	0.047	0.189	0.189	0.189
7	3.23×10^{-4}	0.061	0.061	0.121	0.121	0.121
8	9.97×10^{-5}	0.046	0.091	0.184	0.361	0.361
9	1.58×10^{-4}		0.007			
10	4.89×10^{-4}		0.002			

[a] Abbreviations used: *S.a., Staphylococcus aureus; S.p., Streptococcus pyogenes; C.a., Candida albicans; A.n., Aspergillus niger; T.m., Trichophyton mentagrophytes.*

compound 8 is 0.019% w/v). Compounds 7 and 8 possess appreciable antifungal activity against *Candida albicans*, *Aspergillus niger*, and *Trichophyton mentagrophytes*.

In comparing surface tensions of the equitoxic solutions, as summarized in Table III (excepting compounds **9** and **10**), it is apparent that the concentration of each compound necessary to produce bacterial and fungicidal inhibition reduces the surface tension of water to the same order of magnitude. The mean surface tension of the equitoxic solutions was 59.8 ± 0.3 dyn/cm for bacteria and 51.65 ± 0.1 dyn/cm for fungi, in agreement with the results of Traube,[11] Frobisher,[12] and Zissmann.[13] Previously, James[14] observed that the reduction of surface tension is not in itself bactericidal, since nonionic surfactants are nontoxic. The correlation between equitoxic concentrations and surface tensions is found only with surfactant solutions which are antibacterial. Correlations between the equitoxic concentrations of the compounds and their molecular weights, apparent partition coefficients, and water solubilities were observed.[15] The relatively high thermodynamic activities of the solutions (Table IV) calculated according to the method of Ferguson[16] confirm physical toxicity. On the other hand, compounds **9** and **10** show different thermodynamic activities, which would indicate that they may act through a different mechanism.[17] This is supported by the fact that their equitoxic solutions against *Streptococcus pyogenes* do not present surfactant properties (Table III). Since fatty acids in small amounts are growth factors for *Streptococcus pyogenes*,[18] compounds **9** and **10** (or their hydrolysis products) probably act by inhibiting the mechanism of action of those growth factors. This could be a subject of further research.

Experimental Section

All melting points are uncorrected and were taken on a Büchi capillary melting point apparatus. IR and NMR were determined on a Perkin-Elmer A-700 and a Varian A-60, respectively. A Du Noüy tensiometer was used in the surface-tension experiments. An automatic diluting device (Ames Co., Elkhart, Ind.) was used for MIC determinations. Spectral data and elemental analyses were consistent with the assigned structures. Compounds **4** and **6** were prepared as previously described.[4]

Preparation of N'-(Pyridinioacetyl)alkanoic and -benzoic Acid Hydrazides as Chloride Salts. All were prepared by the same general method, and the procedure is exemplified by the preparation of compound **5**. The products were usually white, crystalline solids, occasionally very hygroscopic (Table I).

N'-(Pyridinioacetyl)decanoic Acid Hydrazide Chloride (5). A solution of 8.0 g (0.03 mol) of N'-(chloroacetyl)decanoic acid hydrazide and 34.3 g (0.43 mol) of dry pyridine was heated in a water bath at 60 °C for 15 min. When the solution cooled, compound **5** crystallized. The solid was removed by filtration and dried in vacuo. Recrystallization from EtOH–AcOEt gave 10.1 g (97.0%): NMR (D_2O) 0.85 (br, 3, methyl protons), 1.25 (s, 14, methylene protons), 2.35 (t, 2, methylene carbonyl protons), 5.65 (s, 1, N-methylene protons), 8.15–8.8 ppm (m, 5, aromatic protons).

Surface Tensions. The ring-detachment method[8] was employed to determine the surface tension of the aqueous solutions of the pyridinium salts synthesized. Water containing 0.1% $KMnO_4$ and 0.1% NaOH was double distilled in an all glass apparatus. Destillate having a surface tension greater than 72 dyn/cm was collected. Several concentrations from 1 to 0.0001%

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w/v were prepared and the surface tensions measured at 22 ± 1 °C. Fourfold analyses were made for each concentration and the mean was taken. The surface tensions plus or minus the standard deviation of 0.1% aqueous solutions are reported in Table I.

Critical Micelle Concentration Determinations. The critical micelle concentrations of the *N'*-(pyridinioacetyl) fatty acid hydrazides have been determined by a surface-tension method.[8] The results were plotted as surface tension against log surfactant concentration. The cmc was interpreted from the intercept of two straight lines (Figure 1).

Antimicrobial Assays. In vitro antimicrobial activity was determined using the autotitration method.[7] The bacteria were inoculated into tryptose phosphate broth (Difco) and incubated at 37 °C for 18–20 h. The fungi were inoculated into proteose peptone no. 3 maltose and incubated at 23 °C for 3–5 days. Absence of growth (turbidity) was indicative of the activity of the drug being evaluated. The lowest level of drug which completely inhibited the development of growth is considered the MIC. Results are summarized in Table II.

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(±)-*trans*-2-(Aminomethyl)cyclobutanecarboxylic Acid Hydrochloride: A Rigid Analogue of γ-Aminobutyric Acid

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The (±) title compound was prepared to evaluate prior observations that certain γ-aminobutyric acid (γAbu) congeners in the transoid disposition demonstrate γAbu receptor binding activity. It was prepared by a multistep sequence from (±)-methyl *trans*-2-(hydroxymethyl)cyclobutanecarboxylate. In a sodium-independent binding assay, the specific binding of (±)-1 to synaptic membranes prepared from rat brain tissue was $^{1}/_{14\,500}$ that of γAbu.

The conformational requirements necessary for biological activity of γ-aminobutyric acid (γAbu) have become the topic of several research studies. Although the conformational requirements of γAbu-ergic compounds have been inferred by physical analysis and theoretical calculations,[1-4] the preparation and subsequent pharmacological testing of structurally rigid molecules containing a segment of the γAbu molecule[5-8] have become the most useful approaches to investigations of this type. These latter studies with the rigid analogues have led to the suggestion that an extended conformation of γAbu is preferred for binding at the receptor and is required for pharmacological activity.[9] In order to further test this hypothesis, we have prepared (±)-2-(aminomethyl)cyclobutanecarboxylic acid hydrochloride (1), a molecule conformationally restricted to resemble the extended form of γAbu. We have tested this agent for its ability to compete with γAbu for sodium-independent synaptic membrane binding sites prepared from rat brain tissue.

Results and Discussion

Synthesis of (±)-1 from (±)-methyl hydrogen-*trans*-cyclobutane-1,2-dicarboxylate (2) is outlined in Scheme I.

Scheme I

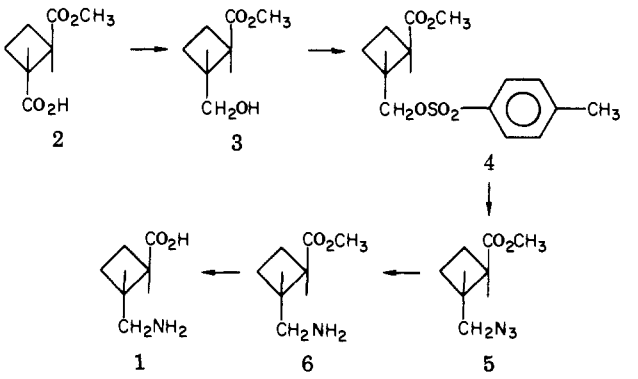

Table I. Sodium-Independent γAbu Receptor Binding in Rat Brain Tissue

agent tested	IC_{50} of [^{3}H]γAbu binding,[a] mol/L	relative affinity
muscimol	3.20 (±0.12) × 10^{-9} [b]	6.3
γAbu	2.00 (±0.03) × 10^{-8} [b]	1
bicuculline	3.80 (±0.27) × 10^{-6} [b]	$^{1}/_{190}$
(±)-*trans*-2-(aminomethyl)cyclobutanecarboxylic acid	2.90 (±0.14) × 10^{-4} [b]	$^{1}/_{14\,500}$

[a] Mean (± SEM) of four to six experiments performed in triplicate. [b] $p = 0.05$ as compared to γAbu.

Reduction of **2** with diborane gave the acid alcohol **3**, which was readily converted to the tosylate **4**. Displacement of the tosylate with sodium azide resulted in the azido ester **5**, which due to its explosive nature was not purified. Reduction of **5** yielded the amino ester **6**, which was subsequently hydrolyzed with hydrochloric acid to the amino acid **1**.

The potency of (±)-1, relative to other γAbu agonists and antagonists, in displacing [^{3}H]γAbu from sodium-independent γAbu binding sites was examined in crude sy-

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