mg/kg a reverse effect was observed: Choline receptor sensitivity was significantly reduced (by 70-77%) and the andrenoreceptor response was amplified by about the same degree, i.e., 70-75%.

Thus, the new ammonium chloride derivatives exhibit distinct cardiotropic properties. Compounds IV and V amplify myocardial contractile properties and exceed the effect of strophanthin with respect to cardiotonic activity and toxicity. Their modulating effect on cardiovascular chemoreceptors also indicates the potential benefit to be gained from a further synthesis of similar compounds for cardiological practice.

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SYNTHESIS, PROPERTIES, AND CARDIOVASCULAR ACTIVITY OF SUBSTITUTED 4-DIHYDROPYRIDINE-2(3H)-THIONES

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In a continuation of our research on the synthesis and study of the properties and biological activity of 1,4-dihydropyridine-2(3H)-thiones [4] we synthesized 4-substituted 6-methyl-5-acetyl-3-cyano-1,4-dihydropyridine-2(3H)-thiones and investigated their cardiovascular activity.

6-Methyl-5-acetyl-3-cyano-1,4-dihydropyridine-2-thiones III and betaines IV were synthesized by three previously described methods. Depending on the substituent in the 4 position and the reaction conditions, the following intermediates were isolated in the condensation of arylideneacetylacetone with cyanothioacetamide [2, 5] (pathway A), in the condensation of acetylacetone, an aromatic aldehyde, and cyanothioacetamide [4] (pathway B), and in the condensation of acetylacetone with 2-cyanoacrylthioamides [5, 6] (pathway C) in the presence of piperidine: piperidinium 6-hydroxy-1,4,5,6-tetrahydropyridine-2-thiolates I, piperidinium 1,4-dihydropyridine-2-thiolates II, or mixtures of them. Thiolates I are unstable compounds and undergo dehydration to give thiolates II on recrystallization. Salt I can be obtained as the principal product only in the case of a strong electron acceptor (R = p-$NO_2C_6H_4$, 4-pyridyl) in the 4 position. Thiones III and betaines IV are formed by brief refluxing of I and II or mixtures of them with an equimolar amount of hydrochloric acid in ethanol. 1,4-Dihydropyridine-2-thione sodium salts V were synthesized by the action of sodium ethoxide on thiones III and betaines IV in order to obtain water-soluble compounds (Table 1).

It was demonstrated by PMR spectroscopy that 4-aryl-substituted 3-cyano-1,4-dihydropyridine-2(3H)-thiones III are formed as mixtures of the cis and trans isomers in a ratio of 1.2:1.0 (Table 2); this differs from the data presented in [7]. The assignment of the iso-

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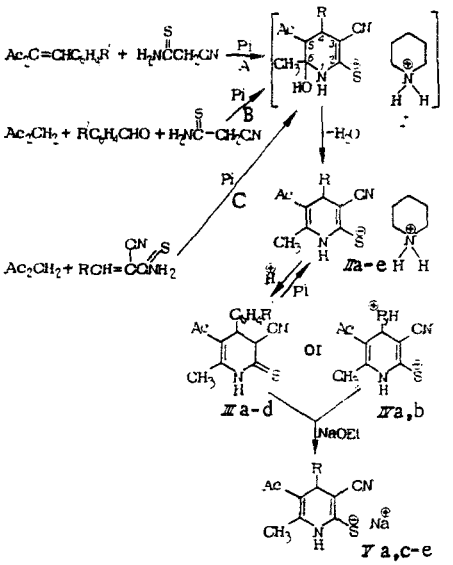
TABLE 1. Characteristics of I-V

Compound	Yield, %	mp, °C	Found, %				Empirical formula	Calculated, %			
			C	H	N	S		C	H	N	S
IIa	64[b]	155—7	67,5	7,0	12,2	9,1	$C_{20}H_{25}N_3OS$	67,6	7,0	11,8	9,0
IIb	62[a], 58[b], 54[d]	113—6	57,4	6,2	13,1	7,5	$C_{20}H_{24}NO_3S\times$ $\times H_2O$	57,1	6,3	13,4	8,0
IIc	60[a], 91[d]	152—4	60,0	6,0	14,0	8,0	$C_{20}H_{24}N_4O_3S$	60,0	6,0	14,9	8,0
IId	61[a], 59[b], 75[d]	116—9	61,0	6,1	10,5	8,3	$C_{20}H_{24}N_3ClOS$	61,6	6,2	10,8	8,2
IIe	59[c], 76[d]	176—8	64,3	6,9	15,6	8,9	$C_{20}H_{24}N_4OS$	64,0	6,8	15,7	9,0
IIIa	60[e]	164—6	67,2	5,5	10,2	12,0	$C_{15}H_{14}N_2OS$	66,7	5,2	10,4	11,9
IIIb	62[e]	159—61	56,9	3,9	13,2	10,2	$C_{15}H_{13}N_3O_3S$	57,1	4,1	13,3	10,2
IIIc	61[e]	172—4	56,7	4,0	12,9	9,9	$C_{15}H_{13}N_3O_3S$	57,1	4,1	13,3	10,2
IIId	60[e]	163—4[f]	59,3	4,5	9,3	10,9	$C_{15}H_{13}N_2ClOS$	59,1	4,3	9,2	10,5
IVa	64[e]	245—8	61,8	4,7	15,1	11,5	$C_{14}H_{13}N_3OS$	62,0	4,8	15,5	11,8
IVb	84[e]	218—20[g]	61,6	4,6	15,4	11,5	$C_{14}H_{13}N_3OS$	62,0	4,8	15,5	11,8
Va	92	176—8[g]	62,0	4,8	9,9	10,6	$C_{15}H_{13}N_2OSNa$	61,6	4,5	9,6	11,0
Vc	53	200—3[g]	52,9	3,4	12,6	9,2	$C_{15}H_{12}N_3OSNa$	53,4	3,6	12,3	9,5
Vd	73	175—80[g]	55,0	3,5	8,5	9,4	$C_{15}H_{12}N_2ClOSNa$	55,1	3,7	8,6	9,8
Ve	91	229—32	56,8	4,1	14,5	10,8	$C_{14}H_{11}N_3OSNa$	57,3	4,1	14,3	10,9

[a]Method A.
[b]Method B.
[c]Method C.
[d]Method D.
[e]The highest overall yield based on cyanothioacetamide.
[f]Literature mp 188-190°C [7].
[g]With decomposition.



II, V, a : R=ph, b : R=p-$NO_2C_6H_4$, c : R=m-$NO_2C_6H_4$, d: R=p-ClC_6H_4, e: R= 4-pyridyl;
III, a : R^1=H, b : R^1=p-NO_2, c : R^1=m-NO_2, d : R^1=p-Cl; IV; a : P_y= 4-pyridyl,
b : P_y= 3-pyridyl; Pi= piperidine.

mers was made in analogy with [5]. The most characteristic bands in the IR spectra of thiones III are the bands of stretching vibrations of a C≡N group at 2248-2261 cm^{-1} and the $\nu_{C=O}$ band of the β-aminovinyl keto fragment with intermolecular hydrogen bonds at 1623-1670 cm^{-1}.

In contrast to 4-aryl-substituted 1,4-dihydropyridine-2-thiones III, 4-pyridyl-substituted 6-methyl-5-acetyl-3-cyano-1,4-dihydropyridine-2-thiones IV exist in the form of betaines [6, 8]. In the IR spectra of betaines IV and salts II and V an absorption band of stretching vibrations of C≡N groups is observed at 2172-2187 cm^{-1} (Table 3), which indicates the extremely pronounced conjugation of the cyano group with the anion. In addition, the IR and PMR spectra of salts II and V and betaines IV contain an absorption band of an NH group and signals of NH protons; this indicates the formation of the anion by detachment of

TABLE 2. PMR Spectra of I-III in $CDCl_3$ and of IV and V in d_6-DMSO

Compound	Chemical shifts, δ, ppm (multiplicity)								KCCB, J, Hz		
	NH (br. s)	NH_2 (br. s)	C_6H_4R or Py (m or d) cis (trans)	$H_{(4)}$ (d) cis (trans)	$H_{(3)}$ (d) cis (trans)	5-$COOH_3$ (s)	6-CH_3 (s or d)	$N(CH_2)_2$ and $(CH_2)_3$ (m and m)	$H_{(3)}-H_{(4)}$ cis (trans)	$CH_3-H_{(4)}$ cis (trans)	$NH-H_{(3)}$ cis (trans)
Ib*	6,38	4,38	8,14 and 7,42	4,05	—	1,73	1,42	3,07 and 1,69	—	—	—
Ie**	6,65	4,28	8,50 and 7,18	3,90	—	1,73	1,42	3,00 and 1,62	—	—	—
IIa	6,51	6,05	7,22	4,50	—	2,01	2,36	2,92 and 1,58	—	—	—
IIb	6,73	5,02	8,10 and 7,36	4,66	—	2,09	2,35	3,02 and 1,64	—	—	—
IIc	6,62	6,62	8,1—7,3	4,62	—	2,10	2,36	3,05 and 1,63	—	—	—
IId	6,49	5,36	7,26 and 7,17	4,51	—	2,03	2,33	2,95 and 1,62	—	—	—
IIe	6,53	5,15	8,24 and 7,13	4,55	—	2,07	2,36	2,92 and 1,65	—	—	—
IIa	8,64	—	7,4—7,1	4,33 (4,39)	4,28 (4,20)	2,10 (2,16)	2,47 (2,52)	—	6,3 (2,6)	0,7 (0,9)	0,7 (0,4)
IIIb	8,61	—	8,23 and 7,40 (8,21 and 7,31)	4,44 (4,51)	4,29 (4,21)	2,16 (2,24)	2,49 (2,55)	—	6,5 (2,2)	0,6 (0,7)	0,9 (0,4)
IIIc	8,78	—	8,2—7,1	4,47 (4,51)	4,28 (4,22)	2,17 (2,27)	2,52 (2,56)	—	6,3 (2,5)	0,7 (0,9)	0,7 (0,4)
IIId	8,69	—	7,35 and 7,16 (7,33 and 7,07)	4,32 (4,36)	4,22 (4,18)	2,11 (2,17)	2,44 (2,49)	—	6,6 (2,8)	0,7 (0,7)	0,6 (0,4)
IVa	6,36	—	8,60 and 7,48	4,50	—	2,02	2,26	—	—	—	—
IVb	5,30	—	8,5—7,5	4,51	—	2,04	2,27	—	—	—	—
Va	8,24	—	7,37 and 7,10	4,34	—	1,95	2,27	—	—	—	—
Vc	8,44	—	8,1—7,5	4,50	—	2,03	2,28	—	—	—	—
Vd	8,33	—	7,33 and 7,13	4,37	—	1,98	2,27	—	—	—	—
Ve	8,30	—	8,38 and 7,06	4,32	—	1,97	2,28	—	—	—	—

*The signal of the OH proton shows up at 5.83 ppm, while that of the $H_{(5)}$ proton shows up at 2.98 ppm with $^3J_{H(4)H(5)}$ 12 Hz.

**The signal of the OH proton shows up at 5.98 ppm, while that of the $H_{(5)}$ proton shows up at 2.95 ppm with $^3J_{H(4)H(5)}$ 12 Hz.

TABLE 3. IR and UV Spectra of II-V

Compound	IR spectra, ν_{max}, cm^{-1}				UV spectra, λ, nm
	C=C	C=O	C≡N	NH	
IIa	1626	1634	2172	3210	278, 322, 381 i
IIb	1639	1667	2180	3246, 3344	274, 332i, 390 i
IIc*	1578	1614 i	2184	3296	271, 324, 380i
IIe	1631	1664	2182	3204	267, 342, 382 i
IIIa	1624	1648	2254	3285, 3322	269,335
IIIb	1626	1645	2248	3200, 3270	272, 334
IIIc	1600 i	1623	2261	3279	266. 336
IIId	1648 i	1670	2250	3210, 3300	219 i, 264, 344
IVa	1620	1641	2176	3224	264, 328, 372 i
IVb	1582	1610	2187	3296	267, 326, 373 i
Va	1650**		2172	3180	250i, 324, 375 i
Vc	1650**		2178	3210, 3340	274, 318, 375 i
Vd	1650**		2178	3205, 3310	257, 325, 367 i
Ve	1648**		2182	3196, 3346	267, 324, 378 i

*In the case of a solution in dioxane we observed $\nu_{C=O}$ at 1665 cm^{-1}, ν_{CN} at 2172 cm^{-1}, and ν_{NH} at 3300 cm^{-1}.
**The absorption band was broadened.

a proton from the tertiary carbon atom of the dihydropyridine-2-thione ring. In contrast to the vicinal two-proton system in thiones III, the most characteristic signal in the PMR spectra of salts II and V and betaines IV is the 4-H singlet at 4.32-4.66 ppm.

The ionization constants in 50% aqueous ethanol solutions were determined. Thiones III are characterized by pK_a 3.17-3.84, while betaines IV are characterized by pK_a 2.01-2.06 and pK_{NH^+} 4.51-5.19. Being a strong electron acceptor, the pyridinium cation appreciably intensifies the acidic properties of IV. The 5-acyl group in thiones III as compared with the 5-alkoxycarbonyl group has a similar effect [6].

Compounds II-V are unstable in dilute solutions and are oxidized by air oxygen both in the hydrogenated ring and at the sulfur atom [5]. Only freshly prepared solutions of III-V were therefore used to study the cardiovascular activity.

Compound IIIc has the most pronounced and prolonged effect on the blood flow in the femoral artery without substantially changing the blood flow in the vessels of the heart (a dose of 0.1 mg/kg increases the blood flow by 50% in 15 min; Table 4). Compound IIId also causes a distinct increase in the blood flow in the femoral artery (a dose of 0.1 mg/kg increases the blood flow by 36% in 30 min). The vasodilating effect of thione IIId on the femoral artery becomes weaker with an increase in the dose, but an effect on the vessels of the heart is observed. Compounds IIId and IIIc in higher doses (1.0 and 0.5 mg/kg, respectively) decrease to a certain degree the frequency of heart contractions and decrease the systemic arterial pressure. In experiments on spontaneously hypertensive rats (SHR) salt Vc increases the arterial pressure without changing the pressure in narcotized animals while manifesting an insignificant vasodilating effect.

Compounds IIId and IVa are the most toxic compounds. The remaining investigated compounds have LD_{50} ~ 1000 mg/kg. Compound IIIc, with a comparatively pronounced vasodilating effect on the vessels of the skeletal musculature that significantly exceeds the effect of Trental, also displays a low acute toxicity.

EXPERIMENTAL (CHEMISTRY)

The IR spectra of suspensions of the compounds in mineral oil (Nujol) and of solutions in dioxane were recorded with a Perkin–Elmer 580B spectrometer (England). The UV spectra of solutions in ethanol were recorded with a Specord UV-VIS spectrophotometer (East Germany). The PMR spectra were obtained with a WH 90/DC spectrometer (West Germany) at 90 MHz with tetramethylsilane as the internal standard. The ionization constants were determined by potentiometric titration with a glass electrode in 50% aqueous ethanol. The principal physicochemical and pharmacological characteristics of the synthesized substances are given in Tables 1-4.

Piperidinium 6-Methyl-4-aryl(piperidyl)-5-acetyl-3-cyano-1,4-dihydropyridine-2-thiolates II. A) A mixture of 10 mmole of arylideneacetylacetone and 10 mmole of cyanothioace-

TABLE 4. Effect of 1,4-Dihydropyridine-2(3H)-thiones III, Betaines IV, and Salts V on the Parameters of the Cardiovascular System and Their Acute Toxicities

Compound	Dose, mg/kg	Increase in the coronary blood flow		Increase in the blood flow in the femoral artery		Change in the pulse frequency, %	Hypotensive reaction, %	Hypotensive reaction in SHR (10 mg/kg), mm of Hg	LD_{50}, mg/kg (intraperitoneally in mice)
		effect, %	duration, min	effect, %	duration, min				
IIIa*	0,01	1	0,5	—	...	1↑	5↑	6↓ (3 h)	1000
	0,1	1	0,5	—	...	1↑	11↑		
	1,0	—		—	...	4↑	20↑		
IIIc	0,1	5	2	50	15	—	—	4↓ (3 h)	1248 (721—2159)
	0,5	55	15	95	45	10↓	15↓		
IIId	0,1	13	7	36	30	—	11↓	...	>100
	1,0	32	30	16	12	13↓	14↓	...	
IVa	0,1	8	3	—	...	—	—	...	500
	1,0	18	5	5	2	—	—	...	
IVb	0,1	5	2	...	...	4↓	4↓	...	>1000
	1,0	—	—	...	...	—	5↓	...	
Va	0,1	9	5	8	15	4↑	6↑	5↑ (1 h)	<1000
	1,0	12	3	7	5	—	—		
Vc*	0,1	15	10	10	5	—	—	8↑ (1 h)	~1000
	1,0	13	5	7	3	—	—		
Trental	0,5	10	5	65	22	—	18↓	...	330 (277—392)

Note. ↓ denotes a decrease in the effect, while ↑ denotes an increase in the effect; the asterisk pertains to experiments on dogs; SHR pertains to spontaneously hypertensive rats.

tamide in 15-25 ml of absolute ethanol and 1 ml (12.5 mmole) of piperidine was stirred for 10-15 min at room temperature, after which it was cooled to 0°C. After 10-30 min, the product crystallized out. It was then removed by filtration and washed with 10-15 ml of cold ethanol. The crude product was recrystallized from ethanol. Compounds IIb-d were obtained in 60-62% yields.

B) A mixture of 10 mmole of acetylacetone, 10 mmole of the aromatic aldehyde, and 10 mmole of cyanothioacetamide in 15-20 ml of absolute ethanol and 1 ml (12.5 mmole) of piperidine was stirred for 3-5 min at room temperature. After 5-20 min, the product crystallized out and was removed by filtration and washed with 10-15 ml of cold ethanol. The crude product was recrystallized from ethanol. Compounds IIa, b, d were obtained in 58-64% yields.

C) A mixture of 10 mmole of acetylacetone and 10 mmole of 2-cyano-3-pyridylacrylthioamide in 20 ml of absolute ethanol and 1 ml (12.5 mmole) of piperidine was stirred for 20-30 min at room temperature. The reaction mixture was then cooled to 0°C, and the precipitate was removed by filtration and washed with 10-15 ml of cold ethanol and 20 ml of ether. The crude product was recrystallized from ethanol. Compound IIe was obtained in 59% yield.

D) A mixture of 5 mmole of thione III or betaine IV and 0.8 ml (10 mmole) of piperidine in 10 ml of absolute ethanol was stirred for 15-30 min at room temperature, after which it was cooled to 0°C. The precipitate was removed by filtration and washed with 5-10 ml of cold ethanol. Compounds II were obtained in 54-91% yields.

6-Methyl-4-aryl-5-acetyl-3-cyano-1,4-dihydropyridine-2(3H)-thiones III and 6-Methyl-5-acetyl-3-cyano-4-pyridyl-1,4-dihydropyridine-2(3H)-thione Betaines IV. The crude product obtained by methods A, B, or C (II or a mixture of I and II) from 10 mmole of the starting substance was dissolved by heating in 20 ml of a 0.5 N solution of hydrochloric acid in ethanol, and the solution was filtered and cooled to 0°C. After 10-20 min, the precipitate was removed by filtration, washed with 5 ml of cold ethanol and 15-20 ml of water, and recrystallized from ethanol. Compounds III and IV were obtained in 60-68% yields.

Sodium 6-Methyl-4-aryl(pyridyl)-5-acetyl-3-cyano-1,4-dihydropyridine-2-thiolates V. A mixture of 5 mmole of thione III or betaine IV and 10 ml of a 0.5 N solution of NaOEt was stirred for 15-20 min at room temperature, after which it was filtered, cooled to 0°C, and

treated with 10 ml of ether. After 15-20 min, the precipitate was removed by filtration. Compounds V were obtained in 52-92% yields.

EXPERIMENTAL (PHARMACOLOGY)

The experiments were carried out on cats of both sexes with masses of 2.3-3.6 kg that had been narcotized with chloralose (90 mg/kg intraperitoneally). The systemic arterial pressure was recorded by an electromanometric method from the common carotid artery. The dp/dt values were calculated by means of a pressure processor (Nihon Kohden). The EKG were recorded in I standard shunting. The space velocity of the coronary blood flow was determined by the method of Kaverina [1], and the blood flow in the femoral artery was determined by means of an MFV-1200 flow meter (Nihon Kohden). All of the recordings were made with an RM-6000 polygraph (Nihon Kohden).

In the experiments on mongrel dogs of both sexes with masses of 13-24 kg that had been narcotized with ethaminal sodium (50 mg/kg intraperitoneally) the systemic arterial pressure and the EKG were recorded as in the experiments on cats.

The substances were dissolved in 50% dimethylacetamide and were introduced intravenously through a cannula inserted into the femoral vein.

Each substance was investigated on three to four animals, and the average results were calculated.

In the experiments on spontaneously hypertensive rats of the Okamoto–Aoki strain [10] that had been kept awake the systolic arterial pressure was determined by plethysmography [9] prior to administration of the substances and 0.5, 1, 3, 6, and 24 h after its administration. Each dose of substance was investigated on three to six rats using aqueous suspensions prepared by means of Tween 80; the suspensions were introduced into the stomach through a probe.

The acute toxicity was investigated on white mongrel mice with masses of 18-24 g. A suspension of the substance in water with the addition of Tween 80 (0.05 ml of 6% Tween per 5 mg of the substance) was introduced intraperitoneally. Each dose was investigated on three to six mice, which were observed for 10 days. The mean lethal dose (LD_{50}) was determined by the method of Litchfield and Wilcoxon.

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