EUR. J. MED. CHEM. — CHIM. THER., 1983-18, N° 2, pp. 155-161

Sila-pharmaca, XXV [1]. Sila-analogues of nifedipine-like dialkyl 2,6-dimethyl-4-aryl-1,4 dihydropyridine-3,5-dicarboxylates, III (*)

Reinhold TACKE ([1]) (**), Anke BENTLAGE ([1]), Robertson TOWART ([2]) (***) and Eike MÖLLER ([2])

(1) *Institut für Anorganische Chemie der Technischen Universität Braunschweig, Pockelsstrasse 4, D-3300 Braunschweig.*

(2) *Pharma-Forschungszentrum der Bayer AG, D-5600 Wuppertal 1.*

Dedicated to Prof. Dr. Otto BAYER in memoriam

(Received August 5, 1982, accepted December 13, 1982)

Summary. — 15 new C/Si-analogue pairs (C-compounds and sila- or disila-substituted derivatives, respectively), which are structurally related to nifedipine, have been synthesized. These and some further C/Si-pairs have been investigated comparatively with respect to their physicochemical and pharmacological properties. Using reversed-phase thin-layer chromatography it was shown that both the sila- and disila-analogues are more lipophilic than the corresponding C-compounds. With respect to the *in vitro* spasmolytic potencies the Si-compounds show approximately similar structure-activity relationships to their carba-analogues. However, in some cases marked differences in *in vivo* effects (cardiovascular and antihypertensive activity) could be demonstrated.

Résumé. — La synthèse de 15 nouvelles paires d'analogues C/Si (composés carbonés et composés mono- ou disiliciés-substitués), dérivant de la structure de la nifédipine, est décrite. Une étude comparative de leurs propriétés physico-chimiques et pharmacologiques, y inclus quelques autres paires d'analogues C/Si à structure semblable, a été effectuée. La chromatographie en couche mince à phases inversées a montré que les composés siliciés sont plus lipophiles que les composés carbonés correspondants. En ce qui concerne leurs propriétés spasmolytiques *in vitro*, les composés siliciés sont caractérisés par des relations structure-activité semblables à celles des analogues carbonés. Au contraire, la comparaison *in vivo* (activité cardiovasculaire et antihypertensive) a mis en évidence, dans quelques cas, des effets pharmacologiques de substitution C/Si très prononcés.

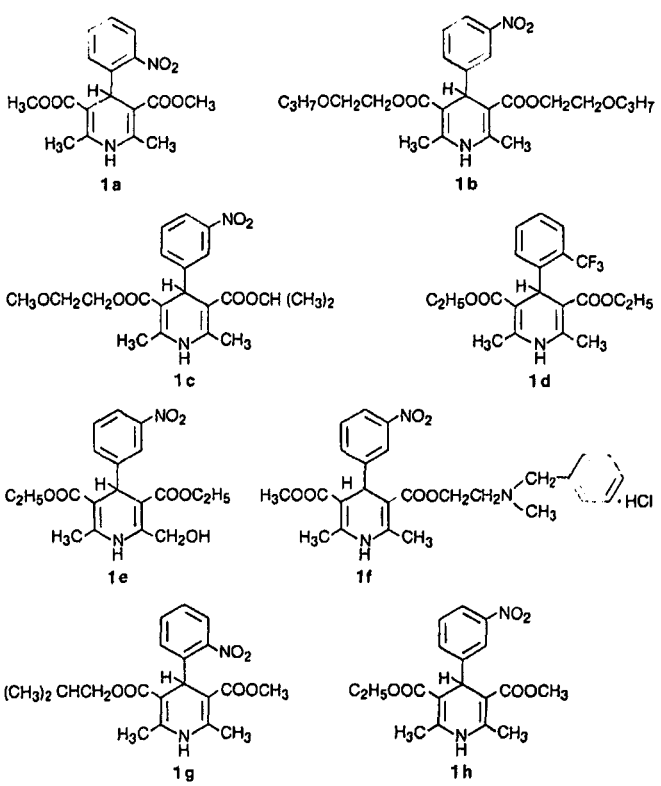
Zusammenfassung. — 15 neue C/Si-Analogenpaare (C-Verbindungen und sila- bzw. disila-substituierte Derivate), die sich strukturell vom Nifedipin ableiten, wurden synthetisiert. Diese und einige weitere C/Si-Paare wurden hinsichtlich ihrer physikochemischen und pharmakologischen Eigenschaften vergleichend untersucht. Mittels reversed-phase-Dünnschichtchromatographie wurde gezeigt, daß die Sila- bzw. Disila-Analoga lipophiler sind als die entsprechenden C-Verbindungen. Bezüglich der spasmolytischen *in vitro*-Aktivitäten zeigen die Si-Verbindungen in erster Näherung ähnliche Struktur-Wirkungs-Beziehungen wie ihre Carba-Analoga. Dagegen konnten hinsichtlich der *in vivo*-Effekte (cardiovasculäre und antihypertensive Aktivität) in einigen Fällen große Unterschiede nachgewiesen werden.

Key-words : Sila-substitution of drugs. — Nifedipine-like C/Si pairs. — Spasmolytic, antihypertensive and cardiovascular activity. — Structure-activity relationships. — Lipophilicity. — R_M-values.

INTRODUCTION

In recent years several highly potent dialkyl 4-aryl-1,4-dihydropyridine-3,5-dicarboxylates have become known, which inhibit the contractility of vascular smooth muscle. Compounds **1a** *(nifedipine, BAY a* 1040*)* (2-12), **1b** *(niludipine, BAY a* 7168*)* (11, 13), **1c** *(nimodipine, BAY e* 9736*)* (14-16), **1d** *(SKF* 24260*)* (17, 18), **1e** *(FR* 7534*)* (19), **1f** *(nicardipine, YC* 93*)* (20), **1g** *(nisoldipine, BAY k* 5552*)* (21), and **1h** *(nitrendipine, BAY e* 5009*)* (22) are particularly active examples of this substance class. Nifedipine is already used in the treatment of ischemic heart disease.

Pharmacological investigations of a number of dialkyl 2,6-dimethyl-4-aryl-1,4-dihydropyridine-3,5-dicarboxylates



(*) Some results have been described previously : R. Tacke, A. Bentlage, R. Towart, and W. Vater, D.O.S. 2 837 477 (13.3.1980) ; Habilitationsschrift R. Tacke, Technische Universität Braunschweig 1980 ; Dissertation A. Bentlage, Technische Universität Braunschweig 1981.

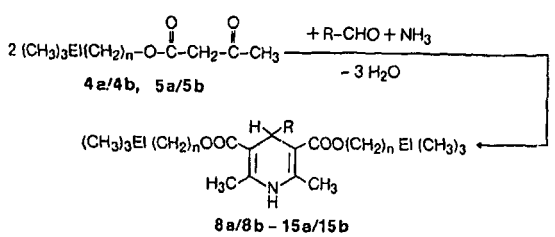
(**) Author to whom correspondence should be addressed.

(***) Present address : Miles Laboratories Ltd., Stoke Court, Slough SL2 4LY, Bucks, U.K.

have shown that the alkyl ester groups in the 3,5-positions of the 1,4-dihydropyridine ring influence the pharmacodynamic and pharmacokinetic properties of these compounds substantially (22, 23). In the course of our work on « sila-pharmaca », it was felt to be of interest to investigate the physicochemical and pharmacological effects of sila-substitution in the alkyl ester groups of nifedipine-like 1,4-dihydropyridines. In two preceding communications (1, 24) we have reported the synthesis and properties of the compounds **13a/13b/13c** and **14a/14b**. The present work describes the synthesis of the 1,4-dihydropyridine derivatives **8a/8b - 12a/12b** and **15a/15b - 24a/24b** (15 new C/Si-pairs) as well as the lipophilic properties and some pharmacological effects of **8a/8b - 24a/24b** and **13c**. Compounds **18a** and **18b** are closely structurally related to nisoldipine (**1g**), and moreover can be regarded as respectively *t*-butyl- and (trimethylsilyl)-substituted derivatives of nifedipine (**1a**). A very pronounced structural similarity also exists between the pair **22a/22b** and nimodipine (**1c**).

CHEMISTRY

The synthesis of the symmetrically 3,5-substituted 1,4-dihydropyridine derivatives **8a/8b - 12a/12b** and **15a/15b** was achieved analogously to the preparation of **13a/13b** and **14a/14b** (cf. lit. (24)) by the reaction of an aldehyde R-CHO with the twofold molar amount of the appropriate alkyl acetoacetate **4a**, **4b**, **5a**, and **5b**, respectively, and an excess of ammonia.

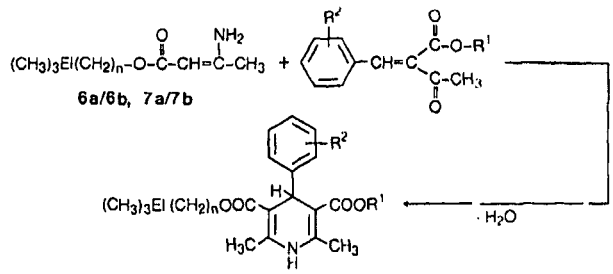


	El	n	R
8a	C	1	C_6H_5
8b	Si	1	C_6H_5
9a	C	1	3-Cl-C_6H_4
9b	Si	1	3-Cl-C_6H_4
10a	C	1	2-Cl-C_6H_4
10b	Si	1	2-Cl-C_6H_4
11a	C	1	3-pyridyl
11b	Si	1	3-pyridyl

	El	n	R
12a	C	1	2-pyridyl
12b	Si	1	2-pyridyl
13a	C	1	3-NO_2-C_6H_4
13b	Si	1	3-NO_2-C_6H_4
14a	C	1	2-NO_2-C_6H_4
14b	Si	1	2-NO_2-C_6H_4
15a	C	2	3-NO_2-C_6H_4
15b	Si	2	3-NO_2-C_6H_4

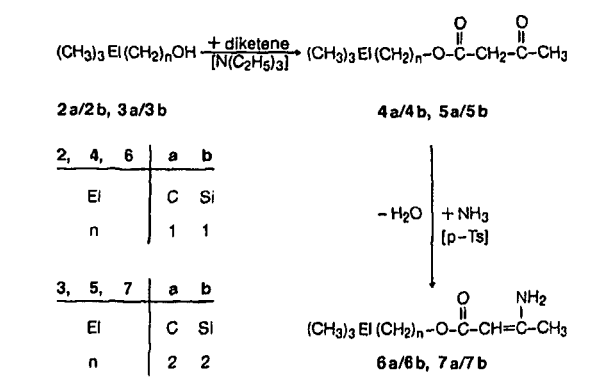
The 3,5-unsymmetrically substituted 1,4-dihydropyridine derivatives **16a/16b - 24a/24b** were synthesized analogously to **13c** (cf. lit. (1) and lit. (25)) by the reaction of the appropriate alkyl β-aminocrotonates **6a**, **6b**, **7a**, and **7b**, respectively, with a 2-aralkylidene-acetoacetate of the type $R^2C_6H_4$-CH=C($COCH_3$)$COOR^1$.

The starting materials **4a**, **4b**, **5a**, and **5b** were prepared in accordance with known methods by the addition of diketene to the appropriate alcohols **2a**, **2b**, **3a** and **3b**, respectively, in the presence of a small quantity triethylamine. By further reaction with ammonia under catalysis



	El	n	R^1	R^2
13c	Si	1	$CH_2C(CH_3)_3$	3-NO_2
16a	C	1	CH_3	4-NO_2
16b	Si	1	CH_3	4-NO_2
17a	C	1	CH_3	3-NO_2
17b	Si	1	CH_3	3-NO_2
18a	C	1	CH_3	2-NO_2
18b	Si	1	CH_3	2-NO_2
19a	C	1	C_2H_5	3-NO_2
19b	Si	1	C_2H_5	3-NO_2
20a	C	1	$CH(CH_3)_2$	3-NO_2
20b	Si	1	$CH(CH_3)_2$	3-NO_2
21a	C	1	CH_2-C≡CH	3-NO_2
21b	Si	1	CH_2-C≡CH	3-NO_2
22a	C	1	$CH_2CH_2OCH_3$	3-NO_2
22b	Si	1	$CH_2CH_2OCH_3$	3-NO_2
23a	C	2	CH_3	3-NO_2
23b	Si	2	CH_3	3-NO_2
24a	C	1	C_2H_5	2-Cl
24b	Si	1	C_2H_5	2-Cl

of *p*-toluene sulfonic acid the alkyl β-aminocrotonates **6a**, **6b**, **7a** and **7b** respectively, may be synthesized, of which **6a** and **6b** have been previously described by us (24).



The structures of the new 1,4-dihydropyridines **8a/8b - 12a/12b** and **15a/15b - 24a/24b** were confirmed by analyses (Table 1 and 2, respectively) and spectroscopic measurements (^{1}H NMR and mass spectroscopy). In all cases the molecular ions M^+ could be established in the mass spectra. The ^{1}H NMR spectra are relatively simple and reflect well the various structural elements of the compounds. The NMR data of 3 C/Si-pairs are listed in Table 3 as typical examples.

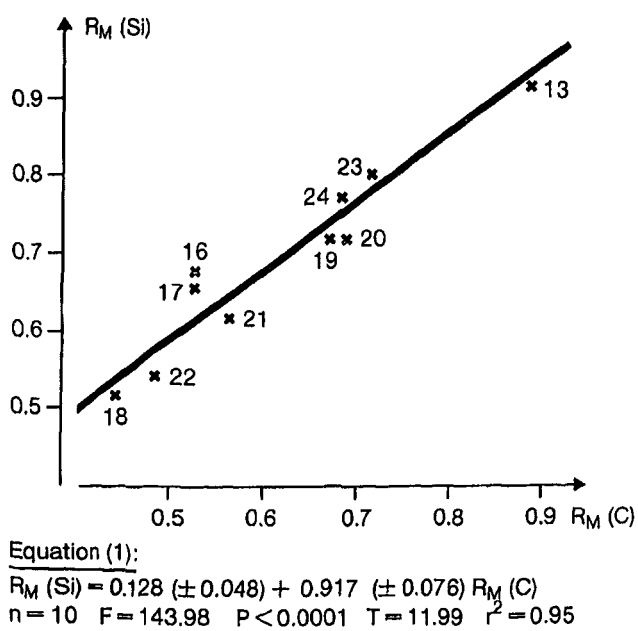
LIPOPHILIC PARAMETERS

Lipophilic parameters are of particular importance in the investigation of quantitative structure-activity relationships of nifedipine analogues (26). The lipophilicity of the compounds **8a/8b - 24a/24b** and **13c** (Table 4) was measured, because substituent constants for a sila-substitution are not known. Instead of the laborious measurement of octanol/water partition coefficients (log P) we measured the R_M-

TABLE I. — *Dialkyl 2,6-dimethyl-4-aryl-1,4-dihydropyridine-3,5-dicarboxylates* **8a/8b - 12a/12b** *and* **15a/15b**

No	Compound	Formula[a] (mol. mass)	Yield[b] [%]	M.p.[c] [°C]	Analyses (calcd./found) [%] C	H	N	Si
8a	Dineopentyl 2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate	$C_{25}H_{35}NO_4$ (413.6)	48	149 (A)	72.61 72.2	8.53 8.6	3.39 3.5	
8b	Bis (trimethylsilyl-methyl) 2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate	$C_{23}H_{35}NO_4Si_2$ (445.7)	46	123 (A)	61.98 62.1	7.91 8.0	3.14 3.2	12.60 12.5
9a	Dineopentyl 2,6-dimethyl-4-(3-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{25}H_{34}ClNO_4$ (448.0)	47	189 (A)	67.03 66.8	7.65 7.6	3.13 3.1	
9b	Bis(trimethylsilyl-methyl) 2,6-dimethyl-4-(3-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{23}H_{34}ClNO_4Si_2$ (480.1)	47	129 (A)	57.53 57.5	7.14 7.2	2.92 3.0	11.70 11.9
10a	Dineopentyl 2,6-dimethyl-4-(2-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{25}H_{34}ClNO_4$ (448.0)	62	187 (B)	67.03 66.8	7.65 7.7	3.13 3.2	
10b	Bis(trimethylsilyl-methyl) 2,6-dimethyl-4-(2-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{23}H_{34}ClNO_4Si_2$ (480.1)	48	129 (A)	57.53 57.5	7.14 7.2	2.92 3.0	11.70 11.6
11a	Dineopentyl 2,6-dimethyl-4-(3-pyridyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{24}H_{34}N_2O_4$ (414.5)	68	172 (B)	69.54 69.5	8.27 8.2	6.76 6.7	
11b	Bis(trimethylsilyl-methyl) 2,6-dimethyl-4-(3-pyridyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{22}H_{34}N_2O_4Si_2$ (446.7)	88	162 (B)	59.16 59.1	7.67 7.7	6.27 6.2	12.57 12.7
12a	Dineopentyl 2,6-dimethyl-4-(2-pyridyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{24}H_{34}N_2O_4$ (414.5)	41	223[d] (B)	69.54 69.4	8.27 8.3	6.76 6.5	
12b	Bis(trimethylsilyl-methyl) 2,6-dimethyl-4-(2-pyridyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{22}H_{34}N_2O_4Si_2$ (446.7)	60	205 (C)	59.16 59.0	7.67 7.6	6.27 6.3	12.57 12.4
15a	Bis(3,3-dimethyl-butyl) 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{27}H_{38}N_2O_6$ (486.6)	67	120 (D)	66.64 66.5	7.87 7.8	5.76 5.8	
15b	Bis(2-trimethylsilyl-ethyl) 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{25}H_{38}N_2O_6Si_2$ (518.8)	42	120 (D)	57.88 58.1	7.38 7.4	5.40 5.3	10.83 11.0

[a]) The molecular masses of all compounds were established by mass spectroscopy.
[b]) The yields are related to recrystallized products and have not been optimized in most cases.
[c]) Average values of several measurements. The recrystallisation solvent is given in parantheses : A ether/petroleum ether, B ethanol/ether/petroleum ether (only a small amount of ethanol as dissolving mediator), C ethanol/methanol, D ether.
[d]) Decomposition.



Equation (1):

$R_M(Si) = 0.128\ (\pm 0.048) + 0.917\ (\pm 0.076)\ R_M(C)$

$n = 10\quad F = 143.98\quad P < 0.0001\quad T = 11.99\quad r^2 = 0.95$

FIG. 1. — Plot of equation (1) showing the linear dependence of $R_M(Si)$ and $R_M(C)$.

TABLE. II — *Dialkyl 2,6-dimethyl-4-aryl-1,4-dihydropyridine-3,5-dicarboxylates* **16a/16b - 24a/24b**

No	Compound	Formula[a] (mol. mass)	Yield[b] [%]	M.p.[c] [°C]	Analyses (calcd./found) [%] C	H	N	Si
16a	Methyl neopentyl 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{21}H_{26}N_2O_6$ (402.4)	70	157 (A)	62.67 62.7	6.51 6.7	6.96 7.0	
16b	Methyl trimethylsilyl-methyl 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{20}H_{26}N_2O_6Si$ (418.5)	58	137 (A)	57.40 56.9	6.26 6.3	6.69 6.8	6.71 6.6
17a	Methyl neopentyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{21}H_{26}N_2O_6$ (402.4)	77	136 (B)	62.67 62.3	6.51 6.8	6.96 7.0	
17b	Methyl trimethylsilyl-methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{20}H_{26}N_2O_6Si$ (418.5)	67	120 (B)	57.40 57.4	6.26 6.2	6.69 6.7	6.71 6.9
18a	Methyl neopentyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{21}H_{26}N_2O_6$ (402.4)	39	173 (B)	62.67 62.5	6.51 6.7	6.96 6.8	
18b	Methyl trimethylsilyl-methyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{20}H_{26}N_2O_6Si$ (418.5)	69	156 (C)	57.40 57.2	6.26 6.3	6.69 6.7	6.71 6.8
19a	Ethyl neopentyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{22}H_{28}N_2O_6$ (416.5)	62	138 (D)	63.45 63.4	6.78 6.8	6.73 6.7	
19b	Ethyl trimethylsilyl-methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{21}H_{28}N_2O_6Si$ (432.5)	55	115 (D)	58.31 58.1	6.52 6.5	6.48 6.6	6.49 6.2
20a	Isopropyl neopentyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{23}H_{30}N_2O_6$ (430.5)	67	153 (B)	64.17 64.3	7.02 7.0	6.51 6.4	
20b	Isopropyl trimethylsilyl-methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{22}H_{30}N_2O_6Si$ (446.6)	85	121 (C)	59.17 59.2	6.77 6.7	6.27 6.3	6.29 6.2
21a	Propin-3-yl neopentyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{23}H_{26}N_2O_6$ (426.5)	83	147 (D)	64.78 64.8	6.14 6.2	6.57 6.3	
21b	Propin-3-yl trimethylsilyl-methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{22}H_{26}N_2O_6Si$ (442.5)	81	148 (D)	59.71 59.7	5.92 6.1	6.33 6.5	6.35 6.5
22a	(2-Methoxy-ethyl) neopentyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{23}H_{30}N_2O_7$ (446.5)	57	124 (D)	61.87 61.5	6.77 6.7	6.27 6.4	
22b	(2-Methoxy-ethyl) trimethylsilyl-methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{22}H_{30}N_2O_7Si$ (462.6)	83	92 (A)	57.12 57.2	6.54 6.6	6.06 5.9	6.07 5.9
23a	Methyl (3,3-dimethyl-butyl) 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{22}H_{28}N_2O_6$ (416.5)	75	97–100[d] (D)	63.45 63.3	6.78 6.8	6.73 6.9	
23b	Methyl (2-trimethylsilyl-ethyl) 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{21}H_{28}N_2O_6Si$ (432.5)	69	125 (D)	58.31 58.0	6.52 6.5	6.48 6.7	
24a	Ethyl neopentyl 2,6-dimethyl-4-(2-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{22}H_{28}ClNO_4$ (405.9)	40	176–179[d] (B)	65.10 64.8	6.95 7.0	3.45 3.5	
24b	Ethyl trimethylsilyl-methyl 2,6-dimethyl-4-(2-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylate	$C_{21}H_{28}ClNO_4Si$ (422.0)	25	119–130[d] (B)	59.77 59.8	6.69 6.7	3.32 3.3	

[a]) The molecular masses of all compounds were established by mass spectroscopy.
[b]) The yields are related to recrystallized products and have not been optimized in most cases.
[c]) Average values of several measurements. The recrystallisation solvent is given in parantheses : A methanol, B ethanol/ether/petroleum ether (only a small amount of ethanol as dissolving mediator), C ethanol/ether, D ethanol (96%).
[d]) The measurements were performed with a Kofler melting point apparatus.

values (see experimental section). The R_M-values of the sila-compounds were then correlated with those of the corresponding C-compounds, resulting in a significant correlation for the exchange of *one* carbon atom by *one* silicon atom [equation (1), Fig. 1] as well as for the replacement of *two* carbon atoms by *two* silicon atoms [equation (2), Fig. 2].

$$R_M(Si) = 0.128\ (\pm 0.048) + 0.917\ (\pm 0.076)\ R_M(C) \qquad (1)$$
$$n = 10,\ F = 143.98,\ P < 0.0001,\ T = 11.99,\ r^2 = 0.95$$

$$R_M(2\,Si) = 0.091\ (\pm 0.054) + 1.060\ (\pm 0.064)\ R_M(C) \qquad (2)$$
$$n = 8,\ F = 278.68,\ P < 0.0001,\ T = 16.69,\ r^2 = 0.98$$

The validity of the regression was confirmed by the following statistical criteria : standard deviation (given in brackets), F-test value (F), level of significance (P), T-test value (T), and squared correlation coefficient (r^2).

TABLE III — *^1H NMR spectroscopic data* [a] *of* **8a/8b, 18a/18b, and 22a/22b.**

No.	Si-CH_3	C-CH_3	>C-C-CH_3	O-CH_3	O-CH_2-Si	O-CH_2-C	C(4)-H	N-H[b]	aromat. H
8a	-----	0.94 s, 18 H	2.35 s, 6 H	-----	-----	3.72 3.83 AB quartet, 4 H J = 11 Hz	5.14 s, 1 H	6.0 s, 1 H	7.0–7.4 m, 5 H
8b	0.05 s, 18 H	-----	2.35 s, 6 H	-----	3.76 s, 4 H	-----	5.07 s, 1 H	6.0 s, 1 H	7.0–7.4 m, 5 H
18a	-----	0.81 s, 9 H	2.29 2.33 s, 3 H s, 3 H	3.59 s, 3 H	-----	3.82 s, 2 H	5.77 s, 1 H	6.0 s, 1 H	7.1–7.8 m, 4 H
18b	0.05 s, 9 H	-----	2.29 2.33 s, 3 H s, 3 H	3.57 s, 3 H	3.79 s, 2 H	-----	5.75 s, 1 H	6.1 s, 1 H	7.1–7.8 m, 4 H
22a	-----	0.88 s, 9 H	2.34 2.42 s, 3 H s, 3 H	3.38 s, 3 H	-----	c) d)	5.19 s, 1 H	6.0 s, 1 H	7.3–8.2 m, 4 H
22b	0.03 s, 9 H	-----	2.33 2.39 s, 3 H s, 3 H	3.36 s, 3 H	3.70 s, 2 H	e)	5.14 s, 1 H	6.2 s, 1 H	7.3–8.2 m, 4 H

[a]) Solvent $CDCl_3$, internal standard $CHCl_3$ (δ 7.27 ppm). Chemical shifts are presented in δ units (ppm), followed by the respective multiplicities and relative intensities.

[b]) Broadened resonance signal.

[c]) OCH_2C : Singlet (1. approximation) at 3.73 (2 H). It is probable that this is an AB-system, whose analysis is complicated by an overlapping with the OCH_2CH_2O-resonance.

[d]) OCH_2CH_2O : AA'BB'-system, centres of the multiplets at 3.58 (2 H) and 4.19 (2 H).

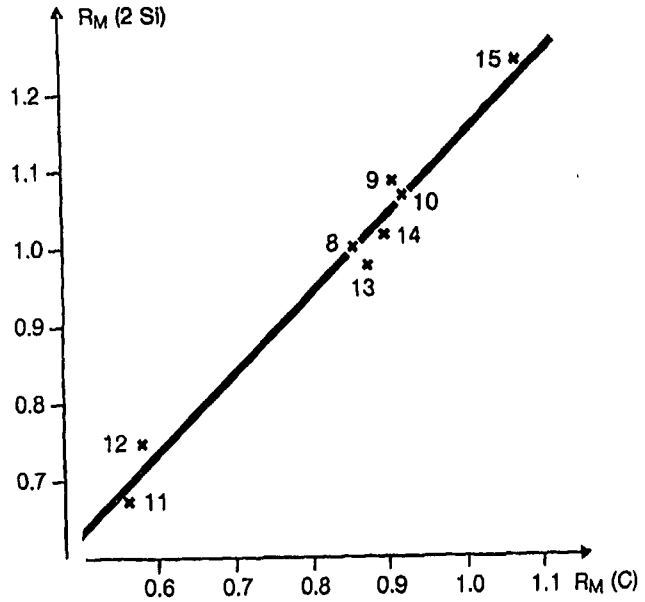
[e]) OCH_2CH_2O : AA'BB'-system, centres of the multiplets at 3.60 (2 H) and 4.20 (2 H).

TABLE IV. — *R_M-values a) of* **8a/8b - 13a/13b, 13c, and 14a/14b - 24a/24b.**

Compound No.	R_M measured	R_M calculated	difference	Compound No.	R_M measured	R_M calculated	difference
8a	0.86			**16b**	0.67	0.62	-0.05
8b	1.00	1.00	0.0	**17a**	0.54		
9a	0.91			**17b**	0.66	0.62	-0.04
9b	1.08	1.06	-0.02	**18a**	0.44		
10a	0.92			**18b**	0.52	0.53	0.01
10b	1.07	1.07	0.0	**19a**	0.67		
11a	0.57			**19b**	0.73	0.76	0.03
11b	0.68	0.7	0.02	**20a**	0.69		
12a	0.59			**20b**	0.73	0.74	0.01
12b	0.75	0.72	-0.03	**21a**	0.56		
13a	0.88			**21b**	0.62	0.64	0.02
13b	0.99	1.02	0.03	**22a**	0.49		
13c	0.93	0.93	0.0	**22b**	0.54	0.58	0.04
14a	0.90			**23a**	0.71		
14b	1.01	1.05	0.04	**23b**	0.8	0.78	-0.02
15a	1.07			**24a**	0.69		
15b	1.25	1.23	-0.03	**24b**	0.77	0.76	-0.01
16a	0.54						

[a]) R_M *measured* : calculated by means of the experimental R_F-values and the formula $R_M = \log [1/R_F - 1]$;
R_M *calculated* : calculated by means of equation (1) and equation (2), respectively.

From equations (1) and (2), it can be concluded that the Si-compounds are more lipophilic than the analogous C-compounds. These results are at first surprising, as one would expect an increase in polarity (and thus a reduced lipophilicity) by sila-substitution, owing to the different electronegativities of carbon and silicon (Allred-Rochow : $\chi_C = 2.50$, $\chi_{Si} = 1.74$). However, these apparently contradictory results may be explained by the fact that the $(CH_3)_3SiCH_2$ group has a greater space filling effect than the corresponding $(CH_3)_3CCH_2$ unit, and thus presents a greater lipophilic surface area. The sila-analogues described here are therefore capable of stronger interactions with the lipid phase.



Equation (2):

$R_M(2\,Si) = 0\,091\ (\pm 0.054) + 1.060\ (\pm 0.064)\ R_M(C)$

$n = 8\quad F = 278.68\quad P < 0.0001\quad T = 16.69\quad r^2 = 0.98$

FIG. 2. — Plot of equation (2) showing the linear dependence of R_M(2Si) and R_M(C).

Similar results have been reported by Woo *et al.* for some sila-substituted barbiturates (27).

From the R_M-values of the C/Si pairs shown in Table 4, a mean increase in lipophilicity $\Delta R_M = 0.07$ for the replacement of *one* carbon atom by *one* silicon atom can be calculated. At present additional experiments are under way to see how far this fragment constant described above may be applicable to compounds of other chemical structures.

PHARMACOLOGY

The spasmolytic properties of **8a/8b - 24a/24b** and **13c** were investigated *in vitro* on the Ba^{++}-stimulated isolated guinea pig ileum. *In vivo* selected compounds were tested with respect to their cardiovascular effects (increase in the coronary sinus oxygen content in the dog after i.v. administration), and to their antihypertensive activity (reduction in the blood pressure on the renal-hypertensive rat after p.o. administration). The results of these pharmacological investigations are given in Table 5.

Discussion of the structure-activity relationships.

The qualitative structure-activity relationships, which derive from the screening data given in Table 5, confirm the results of previous studies carried out in the field of dialkyl 2,6-dimethyl-4-aryl-1,4-dihydropyridine-3,5-dicarboxylates (compare for example lit. (22) and lit. (23)). QSAR-analyses were performed by the use of multiple regression

TABLE V. — *Pharmacological properties* [a,b] *of* **8a/8b - 13a/13b, 13c, 14a/14b - 24a/24b,** *and nifedipine*

Compound No.	Spasmolytic effect[c] (guinea pig, in vitro) ID_{50} (mol/l)	Cardiovascular effect[d] (anaesthetized dog, i.v. administration)	Antihypertensive effect[e] (renal-hypertensive rat, p.o. administration)
8a	5×10^{-7}	+	-----
8b	2×10^{-7}	+	-----
9a	5×10^{-7}	++	-----
9b	7×10^{-7}	++	-----
10a	1×10^{-7}	+	-----
10b	7×10^{-8}	++	-----
11a	$> 1 \times 10^{-6}$	-----	-----
11b	$> 1 \times 10^{-6}$	-----	-----
12a	$> 1 \times 10^{-6}$	+	-----
12b	$> 1 \times 10^{-6}$	+	-----
13a	8×10^{-9}	++	> 100 mg/kg
13b	2×10^{-8}	+	100 mg/kg
13c	6×10^{-9}	+++	10 mg/kg
14a	4×10^{-8}	+	> 100 mg/kg
14b	6×10^{-8}	++	> 100 mg/kg
15a	5×10^{-7}	+	> 100 mg/kg
15b	5×10^{-7}	+	> 100 mg/kg
16a	1×10^{-7}	++	-----
16b	1×10^{-7}	+	-----
17a	2×10^{-9}	++++	1 mg/kg
17b	1×10^{-8}	+++	-----
18a	2×10^{-9}	++++	1 mg/kg
18b	3×10^{-8}	++++	1 mg/kg
19a	3×10^{-9}	++++	3 mg/kg
19b	4×10^{-9}	++++	-----
20a	1×10^{-9}	++++	-----
20b	2×10^{-8}	+++	-----
21a	5×10^{-9}	++++	-----
21b	8×10^{-9}	++++	-----
22a	8×10^{-9}	++++	-----
22b	1×10^{-9}	++++	-----
23a	5×10^{-8}	++	-----
23b	5×10^{-8}	++	-----
24a	5×10^{-8}	++++	-----
24b	5×10^{-8}	+++	-----
nifedipine	1.4×10^{-8}	++++	0.1 mg/kg

[a]) Pharmacological screening data. - [b]) The values for **13a/13b/13c, 14a/14b** and nifedipine are taken from lit. (24). - [c]) The activity is defined by the dose (ID_{50}), which leads to 50 % inhibition of a Ba^{++}-induced contraction of the isolated guinea pig ileum. - [d]) The activity is defined by the dose, which leads to an increase of the O_2-saturation in the coronary sinus blood of at least 20 % : + > 0.5 mg/kg, ++ ≤ 0.5 mg/kg, +++ ≤ 0.05 mg/kg, ++++ ≤ 0.01 mg/kg. The measurement was performed 1 h after administration. - [e]) The activity is defined by the minimum dose which leads to a decrease of the blood pressure of the renal-hypertensive rat of at least 15 mm Hg.

analysis employing steric, electronic and lipophilic parameters, but no significant correlations were observed for the test substances in their entirety. The following trends may, however, be seen for sub-groups : in the series of the symmetrically 3,5-substituted 1,4-dihydropyridines **8a/8b - 15a/15b** the 4-(pyridyl) compounds **11a, 11b, 12a** and **12b** as well as the 4-phenyl derivatives **8a** and **8b** are all of low activity. Similar potencies were found for the 4-(chlorophenyl) compounds **9a, 9b, 10a** and **10b**. The introduction of 4-(nitrophenyl) substituents (**13a, 13b, 14a** and **14b**) leads to a significant increase of the *in vitro* activity, which is comparable with that of nifedipine. However, elongation of both alkyl groups in **13a** and **13b** by a CH_2 group (→ **15a** and **15b**, resp.) decreases the potency. In contrast to the similar *in vitro* activity of **13a, 13b, 14a, 14b**, and nifedipine, these compounds differ substantially in their *in vivo* effects : nifedipine was found to be ~ 1 — 3 orders of magnitude more potent than its bulkier substituted derivatives **13a, 13b, 14a** and **14b**.

The asymmetrically 3,5-substituted 1,4-dihydropyridines **16a/16b - 24a/24b** and **13c** are, *mutatis mutandis*, clearly more potent than the symmetrically substituted compounds. In this series also a decrease of activity was observed when making the alkyl ester groups bulkier (compare **17a** and **17b** with **23a** and **23b**, resp.). In the series of the 4-(nitrophenyl)-1,4-dihydropyridines **16a, 16b, 17a, 17b, 18a** and **18b** the 4'-nitro derivatives **16a** and **16b** are of low activity, whereas the corresponding 2'- und 3'-nitro compounds **17a, 17b, 18a** and **18b** are of 1 — 2 orders of magnitude more potent and reach the activity of nifedipine (*in vitro* and *in vivo*).

Although the potency of some of the compounds described may approach or even exceed that of nifedipine *in vitro* or *in vivo* after i.v. administration (anaesthetized dog) the activity after p.o. administration (renal hypertensive rat) is in general inferior, probably due to the pharmacokinetics.

From the data given in Table 5 it is obvious that the 1,4-dihydropyridine derivatives **8a - 24a** in general exhibit similar qualitative structure-activity relationships *(in vitro)* as their sila-analogues **8b - 24b** : the replacement of one or two carbon atoms in the alkyl ester groups by one or two silicon atoms seems not to change the order of magnitude of the spasmolytic activity. The differences found for the pairs **17a/17b, 18a/18b** and **20a/20b** have to be regarded with reservation (preliminary screening data) and require further investigations. However, in principle small but significant differences between C/Si-analogues are conceivable as shown for the triple **13a/13b/13c** (cf. lit. (24)). With respect to the *in vivo* activity, the sila-substitution effects in general may be more pronounced because of a different behaviour of the analogues concerning absorption, distribution, metabolism, and excretion. For example, careful investigations have shown that the compounds **13a, 13b** and **13c** in spite of their similar *in vitro* activity differ substantially *in vivo* in their antihypertensive potency (24).

The significantly increased lipophilicity caused by the C/Si-exchange in the alkyl ester groups of the 1,4-dihydropyridines tested here seems to be too small to influence the biological activity in a definite manner : so far no correlations between these physicochemical alterations and the observed pharmacological sila-substitution effects could be found.

EXPERIMENTAL SECTION

CHEMISTRY

Melting points were determined on a Mettler FP1 apparatus. ^1H NMR spectra were obtained with a Bruker HFX-90 spectrometer. Mass spectra were recorded on an AEI-MS-9 instrument at 70 eV.

Neopentyl alcohol **2a** and *3, 3-dimethyl-1-butanol* **3a** were commercially available. *(Hydroxymethyl)trimethylsilane* **2b** and *(2-hydroxyethyl)trimethylsilane* **3b** were prepared according lit. 28 and lit.

(29), respectively. *Neopentyl acetoacetate* **4a** and *trimethylsilyl-methyl acetoacetate* **4b** were prepared according lit. (24).

3,3-Dimethyl-butyl acetoacetate **5a**. 8.41 g (0.1 mol) diketene are added dropwise with stirring to 10.22 g (0.1 mol) **3b** and 0.1 mL triethylamine at 90°C, so that the temperature of the reaction mixture remains at 85-95°C without further heating (N_2 atmosphere). The mixture is subsequently stirred for a further 2 h at 90°C and then distilled under vacuum over a Vigreux column. Yield 16.0 g (86 %) of a colourless ^{1}H-NMR-spectroscopic pure liquid. The product is redistilled for analytical purposes. Bp. 65-69°C/0.9 mm (lit. (30) : bp. 223-225°C/760 mm, 108-110.5 °C/11 mm). — ^{1}H NMR ($CDCl_3$) : δ 0.93 (s, 9 H, C-CH_3), 1.56 (m, centre of the AA'-part of an AA'XX'-system, 2 H, C-CH_2-C), 1.94 (« s », >C = C-CH_3, enol form) and 2.27 (s, CO-CH_3, keto form) with a relative total intensity corresponding to 3 H, 3.43 (s, CO-CH_2-CO, keto form), 4.19 (m, centre of the XX'-part of an AA'XX'-system, 2 H, C-CH_2-O), 4.96 (« s », -CH = C<, enol form), OH-resonance could not be observed. — Mass spectroscopy : m/e (M^+) = 186. $C_{10}H_{18}O_3$ (186.3). Calcd. : C 64.49 H 9.74, Found : C 63.9 H 9.6.

2-Trimethylsilyl-ethyl acetoacetate **5b**. Analogous to the preparation of **5a** by the reaction of 70.95 g (0.6 mol) **3b** with 50.44 g (0.6 mol) diketene in the presence of 0.5 mL triethylamine. Yield 94.2 g (77.6 %), bp. 58-61°C/0.3 mm (lit. (31) : bp. 77-80°C/1 mm). — ^{1}H NMR ($CDCl_3$) : δ 0.03 (s, 9 H, Si-CH_3), 0.99 (m, centre of the AA'-part of an AA'XX'-system, 2 H, Si-CH_2-C), 1.92 (« s », >C = C-CH_3, enol form) and 2.24 (s, CO-CH_3, keto form) with a relative total intensity corresponding to 3 H, 3.40 (s, CO-CH_2-CO, keto form), 4.20 (m, centre of the XX'-part of an AA'XX'-system, 2 H, C-CH_2-O), 4.92 (« s », -CH = C<, enol form), OH-resonance could not be observed. — Mass spectroscopy : m/e = 187 ($M^+ - CH_3$). $C_9H_{18}O_3Si$ (202.3). Calcd. : C 53.43 H 8.97, Found : C 53.6 H 8.9.

Neopentyl β-aminocrotonate **6a** and *trimethylsilyl-methyl β-aminocrotonate* **6b** were prepared according lit. (24).

3,3-Dimethyl-butyl β-aminocrotonate **7a**. Ammonia is introduced at a water separator to a solution of 23.28 g (0.125 mol) **5a** and 0.4 g of *p*-toluene sulfonic acid in 200 mL toluene at the boiling temperature until no further water is excreted. The reaction mixture is washed with a dilute aqueous solution of Na_2CO_3 and then with water, and subsequently dried over Na_2SO_4, and the toluene distilled off at 20 mm. The residue is fractionally distilled under vacuum. Yield 19.1 g (82 %) of a colourless ^{1}H-NMR-spectroscopic pure product, which crystallizes in the distillation apparatus. The product is redistilled for analytical purposes. Bp. 92°C/1 mm, mp. 62°C. — ^{1}H NMR ($CDCl_3$) : δ 0.91 (s, 9 H, C-CH_3), 1.56 (m, centre of the AA'-part of an AA'XX'-system, 2 H, C-CH_2-C), 1.86 (« s », 3 H, >C = C-CH_3), 4.10 (m, centre of the XX'-part of an AA'XX'-system, 2 H, C-CH_2-O), 4.50 (« s », 1 H, -CH = C<), NH_2-resonance could not be observed. — Mass spectroscopy : m/e (M^+) = 185. $C_{10}H_{19}NO_2$ (185.3). Calcd. : 64.83 H 10.34 N 7.56, Found : 64.7 H 10.3 N 7.6.

2-Trimethylsilyl-ethyl β-aminocrotonate **7b**. Analogous to the preparation of **7a** by the reaction of 80.93 g (0.4 mol) **5b** with ammonia in 300 mL toluene under catalysis of 0.5 g of *p*-toluene sulfonic acid. Yield 58.3 g (72.4 %), bp. 86°C/0.01 mm, mp. 68°C. — ^{1}H NMR ($CDCl_3$) : δ 0.03 (s, 9 H, Si-CH_3), 0.97 (m, centre of the AA'-part of an AA'XX'-system, 2 H, Si-CH_2-C), 1.90 (« s », 3 H, >C = C-CH_3), 4.15 (m, centre of the XX'-part of an AA'XX'-system, 2 H, C-CH_2-O), 4.50 (« s », 1 H, -CH = C<), NH_2-resonance could not be observed. — Mass spectroscopy : m/e (M^+) = 201. $C_9H_{19}NO_2Si$ (201.3). Calcd. : C 53.69 H 9.51 N 6.96, Found : C 53.7 H 9.5 N 6.9.

Dialkyl 2,6-dimethyl-4-aryl-1,4-dihydropyridine-3,5-dicarboxylates **8a/8b - 12a/12b** and **15a/15b** : 60 mmol alkyl acetoacetate (**4a**, **4b**, **5a**, and **5b**, resp.), 30 mmol of the corresponding aldehyde R-CHO, and 3.3 mL concentrated ammonia, dissolved in 20 mL of ethanol (96 %), are heated to reflux for 20 hours. After cooling and (if necessary) concentrating the solution, the crystallized product is filtered off, recrystallized from the indicated solvent (Table 1), and dried *in vacuo* (yields, physical data and analytical data are given in Table 1).

Dineopentyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate **13a**, *bis(trimethylsilyl-methyl) 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate* **13b**, *dineopentyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate* **14a** and *bis(trimethylsilyl-methyl) 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate* **14b** were prepared according to lit. (24).

Neopentyl trimethylsilyl-methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate **13c** was prepared according to lit. (1).

Dialkyl 2,6-dimethyl-4-aryl-1,4-dihydropyridine-3,5-dicarboxylates **16a 16b - 24a/24b** : A solution of 30 mmol alkyl β-aminocrotonate (**6a**, **6b**, **7a** and **7b**, resp.) and 30 mmol of the corresponding 2-aralkylidene-acetoacetate $R^2C_6H_4$-CH=C ($COCH_3$) $COOR^1$ in 20 mL of ethanol (96 %) is heated to reflux for 20 hours. After cooling and (if necessary) concentrating the solution, the product crystallized (in some cases it is necessary to remove the ethanol completely and to substitute it by the recrystallization solvent, which is described in Table 2). The solid product is filtered off, recrystallized from the indicated solvent (Table 2), and dried *in vacuo* (yields, physical data, and analytical data are given in Table 2).

MEASUREMENT OF LIPOPHILICITY

The relative lipophilicity of the substances was measured by reversed-phase thin-layer chromatography according to the methods described in lit. (32-35). Silanized silica gel plates [E. Merck, Darmstadt, F.R.G., 5747 (60 F_{254})] represented the stationary phase. The polar mobile phase consisted of a dioxane-acetone-water mixture (1 : 2 : 2). All compounds were dissolved in a mixture of methanol-chloroform (1 : 1). 2-3 µl of each solution were applied as a circular spot on the plate by a micropipette. The test substances and two control substances were run from the starting line for about 1 hour at constant temperature (23 ± 0.5°C). After drying the plates, all test spots were detected by UV light at 254 nm. The experiments were repeated eight times. The R_M-values were calculated by means of the experimental R_F-values and the formula $R_M = \log [(1/R_F) - 1]$. Higher R_M-values indicate compounds to be more lipophilic than those of lower R_M-values.

PHARMACOLOGY

8a/8b - 24a/24b and **13c** were investigated with respect to the following pharmacological properties :

1) Spasmolytic effect on the Ba^{++}-stimulated isolated guinea pig ileum (according to the method described in lit. (21)).

2) Variation of the coronary sinus oxygen content in the anaesthetized dog after i.v. administration (according to the method described in lit. (21)).

3) Antihypertensive effect on the renal-hypertensive rat after p.o. administration (according to the method described in lit. (21)).

The *in vitro* pharmacological investigations were carried out under the light of a sodium vapour lamp.

REFERENCES

(1) « Sila-pharmaca, part XXIV » : R. Tacke, A. Bentlage, W.S. Sheldrick, L. Ernst, R. Towart and K. Stoepel, *Z. Naturforsch.*, 1982, **37b**, 443.
(2) F. Bossert and W. Vater, *Naturwissenschaften*, 1971, **58**, 578.
(3) W. Vater, G. Kroneberg, F. Hoffmeister, H. Kaller, K. Meng, A. Oberdorf, W. Puls, K. Schlossmann and K. Stoepel, *Arzneim.-Forsch.* (Drug Res.), 1972, **22**, 1.

(4) K. HASHIMOTO, N. TAIRA, S. CHIBA, K. HASHIMOTO Jr., M. ENDOH, M. KOKUBUN, H. KOKUBUN, T. IIJIMA, T. KIMURA, K. KUBOTA and K. OGURO, *Arzneim.-Forsch.*, (Drug Res.), 1972, **22**, 15.
(5) A. FLECKENSTEIN, H. TRITTHART, H.-J. DÖRING and K.Y. BYON, *Arzneim.-Forsch.* (Drug Res.), 1972, **22**, 22.
(6) W.K. RAFF, F. KOSCHE and W. LOCHNER, *Arzneim.-Forsch.* (Drug Res.), 1972, **22**, 33.
(7) F. KOSCHE, W.K. RAFF and W. LOCHNER, *Arzneim.-Forsch.* (Drug Res.), 1972, **22**, 39.
(8) G. GRÜN and A. FLECKENSTEIN, *Arzneim.-Forsch.* (Drug Res.), 1972, **22**, 334.
(9) T. KOBAYASHI, Y. ITO and I. TAWARA, *Arzneim.-Forsch.* (Drug Res.), 1972, **22**, 380.
(10) R. BAYER, R. RODENKIRCHEN, R. KAUFMANN, J.H. LEE and R. HENNEKES, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 1977, **301**, 29.
(11) A. FLECKENSTEIN, G. FLECKENSTEIN-GRÜN, Y.K. BYON, H.P. HAASTERT and F. SPÄH, *Arzneim.-Forsch.* (Drug Res.), 1979, **29**, 230.
(12) M. HIWATARI, K. SATOH and N. TAIRA, *Arzneim.-Forsch.* (Drug Res.), 1979, **29**, 256.
(13) N. TAIRA, A. NARIMATSU, K. SATOH, N. HIMORI, Y. IMAI and M. HIWATARI, *Arzneim.-Forsch.* (Drug Res.), 1979, **29**, 246.
(14) R. TOWART and S. KAZDA, *Brit. J. Pharmacol.*, 1979, **67**, 409 p.
(15) S. KAZDA, F. HOFFMEISTER, B. GARTHOFF and R. TOWART, *Acta Neurol. Scand.*, 1979, **60** (Suppl. 72), 302.
(16) F. HOFFMEISTER, S. KAZDA and H.P. KRAUSE, *Acta Neurol. Scand.*, 1979, **60** (Suppl. 72), 358.
(17) B. LOEV, S.J. EHRREICH and R.E. TEDESCHI, *J. Pharm. Pharmacol.*, 1972, **24**, 917.
(18) B. LOEV, M.M. GOODMAN, K.M. SNADER, R. TEDESCHI and E. MACKO, *J. Med. Chem.*, 1974, **17**, 956.
(19) S.R. JOLLY and G.J. GROSS, *Eur. J. Pharmacol.*, 1979, **54**, 289.
(20) T. TAKENAKA, S. USUDA, T. NOMURA, H. MAENO and T. SADO, *Arzneim.-Forsch.* (Drug Res.), 1976, **26**, 2172.
(21) S. KAZDA, B. GARTHOFF, H. MEYER, K. SCHLOSSMANN, K. STOEPEL, R. TOWART, W. VATER und E. WEHINGER, *Arzneim.-Forsch.* (Drug Res.), 1980, **30**, 2144.
(22) H. MEYER, F. BOSSERT, E. WEHINGER, K. STOEPEL and W. VATER, *Arzneim.-Forsch.* (Drug Res.), 1981, **31**, 407.
(23) F. BOSSERT, H. HORSTMANN, H. MEYER and W. VATER, *Arzneim.-Forsch.* (Drug Res.), 1979, **29**, 226.
(24) R. TACKE, A. BENTLAGE, R. TOWART, H. MEYER, F. BOSSERT, W. VATER and K. STOEPEL, *Z. Naturforsch.*, 1980, **35b**, 494.
(25) H. MEYER, F. BOSSERT, W. VATER and K. STOEPEL, D.O.S. 2 117 571 (19.10.1972) ; *Chem. Abstr.*, 1973, **78**, 16039q. - H. MEYER, F. BOSSERT, W. VATER and K. STOEPEL, D.O.S. 2 177 572 (19.10. 1972) ; *Chem. Abstr.*, 1973, **78**, 16039r.
(26) R. RODENKIRCHEN, R. BAYER, R. STEINER, F. BOSSERT, H. MEYER, and E. MÖLLER, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 1979, **310**, 69.
(27) D.V. WOO, J.E. CHRISTIAN and R.C. SCHNELL, *Can. J. Pharm. Sci.*, 1979, **14**, 12.
(28) D. SEYFERTH, *J. Amer. Chem. Soc.*, 1959, **81**, 1844.
(29) R.J. FESSENDEN and M.D. COON, *J. Med. Chem.*, 1965, **8**, 604.
(30) J. SOLEIL and V. LOPPINET, *Chim. Ther.*, 1966, **1**, 309.
(31) V.F. MIRONOV, V.P. KOZYUKOV and V.D. SHELUDYAKOV, *Zh. Obshch. Khim.*, 1967, **37**, 1915.
(32) E.C. BATE-SMITH and R.G. WESTALL, *Biochim. Biophys. Acta*, 1950, **4**, 427.
(33) C.B.C. BOYCE and B.V. MILBORROW, *Nature*, 1965, **208**, 537.
(34) I.E. BUSH, *Methods Biochem. Anal.*, 1965, **13**, 1.
(35) E. TOMLINSON, *J. Chromatogr.*, 1975, **113**, 1.