5. Grønvold, F. and Langmyhr, F. J. *Acta Chem. Scand.* **15** (1961) 1949.
6. Westman, S. and Magnéli, A. *Acta Chem. Scand.* **11** (1957) 1587.

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Acetylene Compounds of Potential Pharmacological Value
III *. 4-Dialkylamino-2-butynyl Esters of Benzilic Acid

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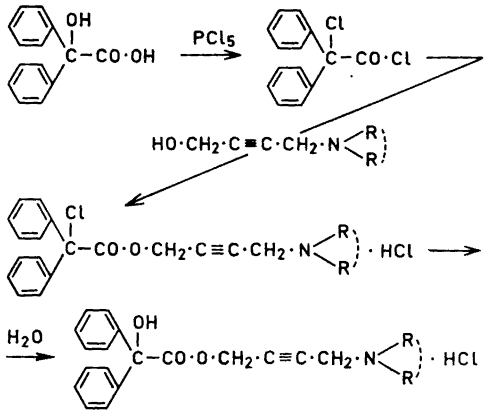
In the first paper of this series [1] a number of 4-dialkylamino-2-butynyl esters of diphenylacetic acid, of 1-phenylcyclopentane-1-carboxylic acid, and of phenothiazine-10-carboxylic acid were synthesised for pharmacological tests on anticholinergic activity and ability to inhibit tremors induced by Tremorine (1,4-dipyrrolidino-2-butyne). As amino esters of benzilic acid often have pronounced anticholinergic activity, we decided to include some 4-dialkylamino-2-butynyl esters of this acid in our investigations.

These esters were prepared in good yield according to a method proposed by King and Holmes [2] for the synthesis of amino esters of benzilic acid, as outlined in the following scheme.



These compounds could also be obtained by trans-esterification of methyl benzilate with the appropriate 4-dialkylamino-2-butyn-1-ol, but this procedure gave much lower yields.

Pharmacology. The hydrochlorides of the 4-alkylamino-2-butynyl benzilates (II, V, VIII, IX) were tested with respect to anticholinergic activity and ability to inhibit tremors induced by 1-(2-oxopyrrolidino)-4-pyrrolidino-2-butyne (oxotremorine). The diethylamino ester II exhibited a strong anticholinergic effect, having about 70 % of the activity of atropine sulphate. The pyrrolidino ester V was also effective in this respect, having 35 % of the activity of atropine sulphate. The piperidino and morpholino esters had only weak activity. The compounds II and V were quite active in the oxotremorine test; a detailed report on this investigation will be published elsewhere. The methobromide III was tested for ganglion-blocking activity. It was about 1–2 times as active as hexamethonium bromide in inhibiting nicotine-induced contractions of the rabbit's ileum.

As the diethylamino ester II bears a certain structural resemblance to the psychopharmacological agent benactyzine (β-diethylaminoethyl benzilate), this compound was compared with chloropromazine with respect to its ability to block conditioned reflex in rats. However, it had little or no effect in this test.

The tests for tremorolytic activity has been carried out by Prof. D. J. Jenden and Dr. R. George, University of California, Los Angeles. All other pharmacological investigations have been performed in the research laboratories of AB Astra, Södertälje, under the direction of Dr. A. Åström.

Experimental. 4-Dialkylamino-2-butynyl esters of α-chlorodiphenylacetic acid. Equimolecular amounts (0.04 mole) of α-chlorodiphenylacetyl chloride [2] and the appropriate 4-dialkylamino-2-butyn-1-ol were mixed, whereupon an exothermic reaction started immediately; the temperature was kept below 50° by external cooling. After a few minutes, the reaction

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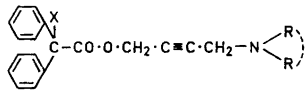
Table 1.



No.	X	-N(R)(R')	Derivative	Yield %	M.p.°C	Formula	Calc. %			Found %		
							C	H	N	C	H	N
I	Cl	$-N(C_2H_5)_2$	HCl	81	96 – 97	$C_{22}H_{24}ClNO_2 \cdot HCl$	65.0	6.20		64.7	6.34	
II	HO	»	»	55	127 – 128	$C_{22}H_{25}NO_3 \cdot HCl$	68.1	6.76	3.61	67.8	6.79	3.41
III	HO	»	CH_3Br	78	149 – 150.5	$C_{22}H_{25}NO_3 \cdot CH_3Br$	61.9	6.32	3.14	61.5	5.76	3.29
IV	Cl	-N□	HCl	78	164 – 165.5	$C_{22}H_{22}ClNO_2 \cdot HCl$	65.4	5.73	3.47	65.4	5.94	3.50
V	HO	»	»	86	137 – 138	$C_{22}H_{23}NO_3 \cdot HCl$	68.5	6.27	3.63	68.3	6.18	3.70
VI	HO	»	Base		110 – 112	$C_{22}H_{23}NO_3$	75.6	6.63	4.01	75.5	6.28	4.05
VII	Cl	-N⬡	HCl	76	141 – 142	$C_{23}H_{24}ClNO_2 \cdot HCl$	66.0	6.02		66.0	6.10	
VIII	HO	»	»	52	146 – 147	$C_{23}H_{25}NO_3 \cdot HCl$	69.1	6.55	3.50	68.7	6.35	3.61
IX	HO	-N⬡O	»	50	148 – 149	$C_{22}H_{23}NO_4 \cdot HCl$	65.7	6.02	3.49	65.7	6.12	3.69

mixture solidified to a glassy mass, which was dissolved in hot acetone (50 ml). On cooling, the *hydrochloride* of the amino ester separated and was recrystallised from ethanol-ether.

The morfolino ester could not be obtained in an analytically pure state, hence the crude product was used as such for synthesis of the α-hydroxy compound.

4-Dialkylamino-2-butynyl esters of benzilic acid. The hydrochloride of the basic esters of α-chlorodiphenylacetic acid, (0.03 mole), prepared as above, was dissolved in water (50 ml) and boiled for 5 min. After cooling, the reaction mixture was made alkaline and the base, which usually crystallised shortly after, was extracted with ether. Addition of dry hydrogen chloride in ether precipitated the *hydrochloride* of the amino ester, which was recrystallised from ethanol-ether.

Trans-esterification of methyl benzilate. The procedure adopted may be illustrated by the following example: Sodium (0.5 g) was dissolved in 4-pyrrolidino-2-butyn-1-ol (7 g), methyl benzilate (2.4 g) was added, and the mixture heated at 50° and 10 mm/Hg for 3 h. Water was added, the pH adjusted to 5, and the solution extracted with ether to remove unreacted methyl benzilate. The aqueous solution was then made alkaline and extracted with ether. Evaporation of the ether yielded crystalline 4-pyrrolidino-2-butyn-1-yl benzilate, which, after recrystallisation from ethanol-water gave white crystals (0.7 g) melting at 110 – 112°.

4-Dimethylamino-2-butyn-1-yl benzilate methobromide (III) was prepared by the addition of methyl bromide to a solution of the free base of II in acetone. The mixture was kept at room temperature overnight, the methobromide precipitated with ether and recrystallised from ethanol-ether.

The physical constants and analytical data of the compounds prepared are collected in Table 1. Infrared spectra were run on a Perkin-Elmer Infracord spectrophotometer, using KBr discs. The spectra were in accordance with those expected of the compounds having the structures presented in Table 1. Melting points (uncorrected) were determined with an electrically heated metal block, using calibrated Anschütz thermometers. Microanalyses were carried out by Dr. A. Bernhardt, Mülheim. The compounds were dried at 50° and 0.05 mm Hg before analysis.

1. Dahlbom, R. and Mollberg, R. *Acta Chem. Scand.* **17** (1963) 916.
2. King, F. E. and Holmes, D. *J. Chem. Soc.* **1947** 164.

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On the Neutral Glycolipids of Human Kidney

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Studies of glycolipids have mostly been limited to the CNS, but some of the glycolipids of spleen,[1,2] erythrocytes,[3,4] and liver [5] have been isolated and described. The neutral glycolipids of blood serum have also recently been studied.[6] From these studies it seems probable that the neutral glycolipids in hitherto studied organs outside the CNS consist mainly of four different sphingolipid fractions, *viz.* ceramide-mono-, di-, and trihexosides and an aminoglycolipid fraction. Except the neutral glycolipids the presence of acid lipids containing sulfate [7] and sialic acid [8] has been established.

Informations about these lipids in normal kidneys are scanty. An immunologically active aminoglycolipid has, however, been isolated by Rapport, Graf and Schneider.[9] It thus seemed appropriate to isolate and describe the different glycolipid fractions of human kidney. A preliminary description of the sulfate containing lipids of this organ has recently been given.[10]

Human kidneys, showing no gross abnormalities, were taken at autopsies from patients aged 60 to 90 years, 24 to 48 h after death. The first steps of the preparation — removal of blood, homogenization, freeze-drying, lipid extraction, mild alkaline hydrolysis of the lipids, and chromatography of the alkalistable lipids on silicic acid columns — were performed exactly as earlier described.[10] The effluent from the silicic acid columns was divided into five fractions; one containing mainly the free fatty acids released by the alkaline hydrolysis, three crude glycolipid fractions, and finally one fraction containing the sphingomyelins with only traces of glycolipids. Thin layer and paper chromatography showed the presence of four main glycolipid components, which had been partially separated. Each of the three crude glycolipid fractions was passed through a DEAE-cellulose column, whereby the acid lipids were removed. The four neutral glycolipid fractions (I—IV) were then isolated by rechromatography on silicic acid columns in chloroform-methanol (C—M) mixtures followed by preparative thin layer chromatography on 1 mm thick layers of silica gel G, C—M—W 65:25:4 (by vol.). Two of the fractions, (I and II) — later shown to be the ceramide-monohexosides and the ceramide-dihexosides — were, however, still heavily contaminated. The monohexosides were, therefore, further purified by chromatography on Florisil in C—M 2:1 (v/v) and then rechromatographed on silicic acid in C—M 9:1 (v/v). The dihexsosides were purified by chromatography on silicic acid in C—M 4:1 (v/v).

The four fractions contained by now 70.1 % of the hexose in the original crude glycolipid fractions. (7.2 % was in the acid lipid fraction, 5.6 % was represented by an unidentified glycolipid, 7.3 % had been taken for analyses, 1.8 % was in the form of smaller mixed fractions not worked up, and 8.2 % were losses not accounted for). Before the final analyses the lipids were dissolved in small volumes of hot methanol and precipitated in the cold.

The analytical results are shown in Table 1. The presence of sphingosine bases in the fractions was demonstrated by paper and thin layer chromatography.* [11,12]

In regard to the analytical data the four fractions can be identified as ceramide-monohexosides, ceramide-dihexosides (glu-gal 1:1), ceramide-trihexosides (glu-gal 1:2) and amino-glycolipids(glu-gal-galam 1:2:1). The glucose-galactose ratios are only approximative, obtained by a subjective valuation of colour intensities of paper chromatogram spots. The R_F-values of the fractions at thin layer chromatography on silica gel G, solvent: C—M—W 65:25:4 (by vol.) [13] and at paper cromatography, solvent: tetrahydrofuran-di-isobutylketone-water 45:5:6 (by vol.),[14] were also the same as for corresponding lipids isolated from blood serum.**

From 542 g freeze-dried kidney tissue was isolated 115 mg monohexosides (I), 223 mg dihexosides (II), 568 mg trihexosides (III), and 1003 mg aminoglycolipides (IV). These weights were obtained before precipitation from methanol, when

* These analyses have kindly been performed by Dr. K.-A. Karlsson of this institute.

** These substances were gifts from Dr. L. Svennerholm of this institute.