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The Effects of Alkyl Substitution in Drugs—III*. The Spasmolytic Activity of Alkyl-Substituted Xanthene-9-Carboxylic Esters

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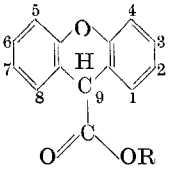
In a previous article, Harms and Nauta[1] discussed the changes in the pharmacological properties of a compound upon the introduction of an alkyl substituent.

Such an effect was demonstrated in substituted dialkylaminoethyl benzhydryl ethers, alkyl substitution at the *ortho* position(s), for example, causing the antihistamine action to diminish while the antiacetylcholine activity increased.

In a series of articles,[2-6] we reported on the spasmolytic activity of a number of mandelic, phenylacetic, diphenylacetic, α-chlorophenylacetic, phenylglyoxylic, benzilic and hexahydrobenzilic acid esters. In this series we limited ourselves, however, to variations in the alcohol moiety and did not consider compounds with substituents in the phenyl groups.

In an effort to combine both lines of investigation, we have synthesized a series of xanthene-9-carboxylic acid esters (I) as analogues of the diphenylacetic acid ester series, and have included some of the alkyl substituted derivatives to find out whether the expected spasmolytic activity would increase when alkyl substituents were introduced.

(I)



* For Part II of this series see This Journal, **2**, 147 (1960).

We have also included a number of quaternized aminoalkyl esters of the alkyl substituted xanthenecarboxylic acids, to compare their pharmacological properties with those of the corresponding esters of the unsubstituted xanthene-9-carboxylic acid, reported by Cusic and Robinson[7] to display considerable parasympatholytic activity. Of this group, the esters of xanthenecarboxylic acid with methyl diethyl-2-oxyethylammonium bromide (methanthelinium bromide*)[8] and with methyl diisopropyl-2-oxyethylammonium bromide (propantheline bromide†)[9] are used clinically.

The alkylxanthene-9-carboxylic esters were prepared from alkylxanthones, which were reduced and carboxylated to give alkylxanthene-9-carboxylic acids. From these, some nitrogen-free esters and a few alkylaminoethanol esters were prepared, the latter being quaternized with methyl bromide.

Experimental

Alkylxanthones. The alkylxanthones were generally prepared according to Ullmann's method,[10,11] although 2,7- and 4,5-dimethylxanthone were prepared using other procedures described in the literature.[12,13] The 1,4-dimethylxanthone, which is not described in the literature, was obtained by Ullmann's method. The ring closure reaction of the intermediate product, 2,5-dimethyl-2′-carboxydiphenylether (m.p. 142°), was effected using acetyl chloride. Crystallization from ligroin gave 1,4-dimethylxanthone as fine white needles, m.p. 129°.

Anal. Calcd. for $C_{15}H_{12}O_2$: C, 80·36; H, 5·36. Found: C, 80·11; H, 5·36.

Alkylxanthenes. The alkylxanthenes were obtained by reduction of the alkylxanthones with 2·5 equivalents of sodium in boiling ethanol,[14] and were isolated by pouring the reaction mixture into water. McConnel[15] has synthesized 2- and 4-methylxanthene and a few of their derivatives described below.

The 1,4-dimethylxanthene was purified by distillation (b.p. 135–140°/0·5 mm).

Anal. Calcd. for $C_{15}H_{24}O$: C, 85·71; H, 6·67. Found: C, 85·34; H, 6·64.

* Banthine ®. † Pro-banthine bromide ®.

Alkylxanthene-9-carboxylic acids. The alkylxanthene-9-carboxylic acids were prepared according to the method described by Burtner and co-workers,[16] alkylxanthene being carboxylated by pouring a solution of alkylxanthyllithium, prepared from alkylxanthene and butyllithium,[17] on to dry solid carbon dioxide. The resulting xanthenecarboxylic acids were crystallized from dilute ethanol. Further data on these acids are listed in Table I.

Table I. Alkylxanthene-9-carboxylic acid

Substituent	Yield, %	m.p., °C	Calcd.		Found	
			C	H	C	H
2-methyl	64	196	74·99	5·04	75·20	5·13
4-methyl	71	204	74·99	5·04	75·12	5·15
1,4-dimethyl	50	213	75·59	5·51	75·24	5·57
2,7-dimethyl	34	238[a]	75·59	5·51	75·44	5·62
4,5-dimethyl	40	241	75·59	5·51	75·60	5·74
1-methyl-4-isopropyl	50	189	76·59	6·37	76·72	6·49

[a] Cusic and Robinson[18]: 226–230°.

1,2,3,4,4a,9a-Hexahydroxanthene-9-carboxylic acid was prepared by hydrogenation of xanthene-9-carboxylic acid (5 g) using 5 per cent rhodium on carbon (10 g) (Baker & Cy., New York, USA) in glacial acetic acid. The theoretical quantity of hydrogen was absorbed during 11 h. This acid crystallized from ethanol in white needles, m.p. 217–218·5°.

Anal. Calcd. for $C_{14}H_{16}O_3$: C, 72·39; H, 6·94. Found: C, 72·37; H, 6·90.

Nitrogen-free esters of alkylxanthene-9-carboxylic acid. These esters were prepared according to the method described by Clinton and Laskowski.[19] The relevant data are given in Table II.

Dialkylaminoethyl esters of alkylxanthene-9-carboxylic acids. These esters were obtained by boiling equivalent amounts of alkylxanthene-9-carboxylic acid and dialkylaminoethyl chloride in isopropyl alcohol under reflux for 4 h.[20] The majority of the resulting hydrochloride salts of the aminoesters could be crystallized from absolute ethanol–ether, after which the free bases were liberated with alkali.

Table II. Nitrogen-free esters of alkyl substituted xanthene-9-carboxylic acids

Alcohol component	Substituents in the ring(s)	b.p., °C/mm	Yield, %	Calcd.		Found		Mol. weight[a]		Spasmolytic activity[b] against spasms elicited by:		
				C	H	C	H	Calcd.	Found	Acetyl-choline	Histamine	$BaCl_2$
2-Methylbutanol-1	none	180/2–3	71	77·00	6·76	77·16	7·07			78	64	55
	2-methyl	165/3	74					310	325	114	50	70
	4-methyl	165/3	77					310	321	121	<15	<10[c]
	1,4-dimethyl	160/0·1	50					324	336	<2·5	<2·5	<2·5[c]
	1-methyl-4-isopropyl	170/0·1	71					352	345	0	0	0[c]
Benzyl alcohol	none	200/2–3	56	79·75	5·01	79·98	5·25			92	39	50
	2-methyl	180–195/0·1	45					330	342	130	<30	<30[c]
	4-methyl	210/0·5	70					330	338	162	27	45
	1,4-dimethyl	170–190/0·5	79					344	334	75	93	88
	1-methyl-4-isopropyl	160–180/0·5	92					372	389	<4	<30	<30[c]
3,3,5-Trimethyl cyclohexanol-1	none	170/0·5	83	78·86	7·43	78·69	7·83			0	0	0[c]
	2-methyl	190–195/0·1	88					364	363	636	<30	63
	4-methyl	197/0·5	66					364	376	23	13	33
	1,4-dimethyl	190–210/0·5	60					378	388	72	69	100
	1-methyl-4-isopropyl	190–200/0·5	77					405	405			

[a] Determined by means of saponification and subsequent titration.
[b] Relative to the 3,5,5-trimethylcyclohexyl mandelate (Cyclospasmol, activity = 100).
[c] The solubility of these compounds is so poor that they are (partly) precipitated in the test vessel. Both the esters and Cyclospasmol were dissolved in propyleneglycol.

By reacting the free aminoesters with methyl bromide in chloroform, ether or benzene (in some cases above room temperature) quaternary salts of these esters were obtained, which, if necessary, were crystallized with the aid of acetone and ether after the solvent had been distilled off. Table IV summarizes the data concerning these esters and their preparation.

Pharmacological Results

The spasmolytic activities of the compounds were determined on the isolated intestine of guinea pig by the method previously described.[21] For the nitrogen-free esters, the activity of the 3,5,5-trimethylcyclohexyl mandelate (Cyclospasmol) was taken as 100; for the nitrogen-containing esters we assigned the value of 100 to propantheline bromide as the standard. The results are listed in Tables II and IV, together with some other data.

The pharmacology of diisopropylaminoethyl 4-methylxanthene-9-carboxylate methylbromide, which displayed a parasympatholytic activity only slightly inferior to that of the unsubstituted compound, was studied in greater detail. A survey of the experiments carried out is presented in Table III.

Table III. Pharmacological activities of diisopropylaminoethyl 4-methylxanthene-9-carboxylate methyl bromide (I) compared with those of the ring unsubstituted compound (II)

Method according to	Aspect studied	(I)	(II)
	LD_{50} intraperitoneally in mice	65 mg/kg	85 mg/kg
	Effect on heart and respiratory frequency in cats	insignificant	insignificant
	Hypotensive effect	100	100[a]
Pulewka[22]	Mydriatic action in mice	100	100[a]
Winbury *et al.*[23]	Chromodacryorrhea in rats (counteraction)	50	100[a]
Brown *et al.*[24]	Antisialagogue action in rabbits	85	100[a]
Fakstorp *et al.*[25]	Ganglion blocking action (nictitating membrane of cat)	70	100[a]
Pradhan *et al.*[26]	Curare effect (sciatic nerve of cat)	80	100[a]
Münchow[27]	Anti-ulcus action in Shay rats. Ulcus number	15	10·5[b]

[a] The activity was arbitrarily put equal to 100.
[b] Subcutaneous injection of 2·5 mg/kg immediately after ligature of pylorus. Rats killed after 18 h. Ulcus number of untreated rats: 28·5.

Table IV. Aminoesters of

Substituent in xanthene-9-carboxylic acid	Hydrochlorides of dialkylaminoethyl esters			
	Yield, %	m.p., °C	Addition of methyl bromide[a]	Yield calcd. on acid, %
Esters of diisopropylaminoethanol				
None	Commercial product			
2-Methyl	85	163–165	C; 80°; 16 h	55
4-Methyl	73	138	C; 80°; 45 h	22
1,4-Dimethyl	100	159–161	C; 80°; 16 h	35
2,7-Dimethyl	70	129–131	C; 65°; 16 h	53
4,5-Dimethyl	100	155–157	C; 80°; 16 h	56
1-Methyl-4-isopropyl		oil	B; 75°; 8 h	17
Hexahydro			C; 60°; 15 h	
Esters of diethylaminoethanol				
None	Commercial product			
2-Methyl		oil	A; 20°; 16 h	17
4-Methyl	80	139	A; 20°; 16 h	68
1,4-Dimethyl	100	164–165	A; 20°; 16 h	52
2,7-Dimethyl	79	136–140	C; 65°; 16 h	57
4,5-Dimethyl	95	169–171	C; 80°; 16 h	51
1-Methyl-4-isopropyl	71	145–148	A; 20°; 16 h	38
4-Methyl	80	139	C; 80°; 45 h[c]	20

alkylxanthene-9-carboxylic acids

Quaternized dialkylaminoethyl esters

m.p., °C	Molecular formula	Analysis, % Calcd. C	Calcd. H	Found C	Found H	Antiacetylcholine activity
						100
[b]	$C_{24}H_{32}BrNO_3.2H_2O$	57·83	7·41	57·83	6·87	33[b]
163	$C_{24}H_{32}BrNO_3.H_2O$	60·00	7·14	59·81	7·26	85
183	$C_{25}H_{34}BrNO_3$	63·03	7·14	62·78	7·13	35
164	$C_{25}H_{34}BrNO_3$	63·03	7·14	63·01	7·53	1
175–177	$C_{25}H_{34}BrNO_3$	63·03	7·14	62·58	7·28	60
151–153	$C_{27}H_{38}BrNO_3$	64·29	7·54	62·81	7·52	0·25
155–160	$C_{23}H_{36}BrNO_3.2·5H_2O$	55·31	8·28	55·18	8·55	2·5
						20
[b]	$C_{22}H_{28}BrNO_3.5H_2O$	50·38	7·31	50·45	7·13	1[b]
183	$C_{22}H_{28}BrNO_3.H_2O$	58·40	6·70	58·41	6·86	46
177	$C_{23}H_{30}BrNO_3$	61·61	6·74	61·20	6·81	31
152[d]	$C_{23}H_{30}BrNO_3.0·5H_2O$	60·39	6·83	60·63	6·74	0·15
[b]	$C_{23}H_{30}BrNO_3.H_2O$	59·23	6·92	59·22	6·90	20[b]
151–153	$C_{25}H_{34}BrNO_3$	63·03	7·14	62·98	7·21	0·85
161	$C_{23}H_{30}BrNO_3$	61·61	6·74	61·69	6·74	50

[a] The addition of methyl bromide was effected in ether (A), benzene (B) or chloroform (C).
[b] These compounds are highly hygroscopic, so that the melting point was not sharply defined. Many of these substances tend to lose their activity in aqueous solution so it is possible that the activities stated are too low and should therefore be regarded as minimum values.
[c] The bromide used in this case was ethyl bromide.
[d] Cusic and Robinson[18]: 151·5–153°.

Discussion

The results do not show a consistent effect of alkyl substitution in xanthene esters, partly due to the poor solubility of the nitrogen-free esters in the test media.

In nearly all cases the already slight antihistamine activity of the esters is decreased still further by substitution. The anti-acetylcholine activity, however, is generally increased upon the introduction of a 2-methyl-substituent, while a 4-methyl group is often also beneficial. Disubstitution (1-methyl-4-isopropyl and 1,4-dimethyl) again reduces the spasmolytic activity. In the series of quaternized aminoesters, propantheline bromide was the most active compound of all the derivatives tested, and although different substitutions evidently affected the activity, no general trends were observed. In the diisopropylaminoethyl series, substitution resulted in a decreased activity, disubstitution with one substituent in both rings being especially unfavourable. In the diethylaminoethyl series, the presence of a 4-methyl group seemed to impart a higher activity to the compound in question, although again disubstitution was less favourable than mono-substitution.

Although, owing to difficulties of synthesis, no compounds with only one substituent in the 1-position were investigated, the results obtained with the 1,4-disubstituted compounds did not seem to show any close parallelism with the diphenylmethyl derivatives, in which *ortho*-alkyl-substitution markedly enhances anticholinergic activity.

Summary. A description is given of the synthesis and spasmolytic activity of a number of xanthene-9-carboxylic esters. The activity of diisopropylaminoethyl 4-methylxanthene-9-carboxylate methyl bromide is compared with that of the ring unsubstituted compound.

Although the activities of a number of these esters increased upon introduction of a methyl group, no general conclusions regarding the relationship between structure and activity could be drawn for this group of compounds.

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