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Study of the cellular action of drugs with protozoa—III. Comparison of the effect of SKF 525-A and related compounds on the multiplication of *Ochromonas danica**

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THE diethylaminoethanol ester of diphenylpropylacetic acid (SKF 525-A) as well as its analog SKF 3301-A inhibited the metabolism of unsaturated fatty acids in the phytoflagellate *Ochromonas danica*[1] and the synthesis of cholesterol in mammals.[2] The purpose of this study is to compare the effects of several analogs of SKF 525-A on *O. danica* to determine (1) if the analogs act on the same metabolic site as SKF 525-A and (2) what part of the molecule is necessary for inhibition.

EXPERIMENTAL

The organism used was *O. danica* Pringsheim. The methods for studying inhibition of multiplication and its annulment have been described.[3, 4] Chemicals were purchased from commercial sources. Fatty acids (99% pure by gas–liquid chromatography) were purchased from the Hormel Institute, Austin, Minn. The SKF compounds: 525-A, 3301-A, 16467-A, 7732-A_3, and 799-$7A_3$ were generously supplied by Dr. W. L. Holmes, Smith, Kline and French Laboratories, Philadelphia, Pa. SKF 2314 was dissolved in 95% ethanol and the other SKF compounds in distilled water. Experiments reported are typical of a minimum of three separate trials giving the same results.

RESULTS

The concentrations of analogs of SKF 525-A causing a 50 per cent inhibition of multiplication are shown in Table 1. The most active compounds were SKF 525-A and its acid SKF 2314 which has

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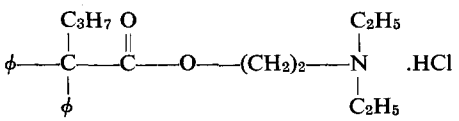
TABLE 1. CONCENTRATION OF SKF 525-A AND RELATED COMPOUNDS GIVING 50 PER CENT INHIBITION OF MULTIPLICATION

Compound	Conc. (mM)*
SKF 525-A	0·05
SKF 2314	0·07
SKF 3301-A	0·15
SKF 16467-A	0·84
SKF 7997-A_3	NI to 1·44 mM†
SKF 7732-A_3	NI to 1·44 mM
Dimethylaminoethanol	NI to 8·5 mM
Diethylaminoethanol	NI to 11·2 mM
3-Dimethylamino-1-propanol	NI to 9·4
3-Diethylamino-1-propanol	NI to 7·6

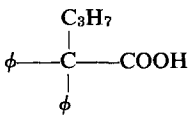
* This concentration is somewhat variable. Larger inocula of microorganisms require higher concentration of drug for 50 per cent inhibition.

† NI = not inhibitory.

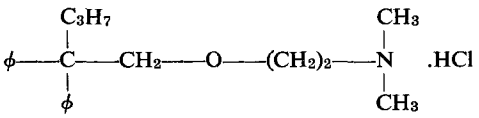
the diphenylpropylacetic acid moiety but lacks the diethylaminoethanol of SKF 525-A (Fig. 1). Compounds like diethylaminoethanol or containing the diethylaminoethanol (SKF 7997-A_3) or dimethylaminoethanol (SKF 7732-A_3) moiety were inactive at the concentrations tested.



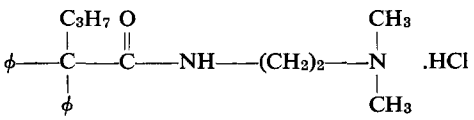
β-Diethylaminoethyl-diphenylpropyl acetate·HCl (SKF 525-A)



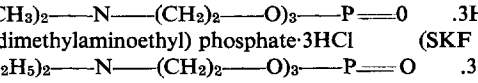
Diphenylpropylacetic acid (SKF 2314)



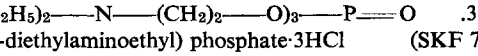
2,2-Diphenyl-1-(β-dimethylaminoethoxy) pentane·HCl (SKF 3301-A)



β-Dimethylaminoethylamino-diphenylpropyl acetate·HCl (SKF 16467-A)



Tris (2-dimethylaminoethyl) phosphate·3HCl (SKF 7732-A_3)



Tris (2-diethylaminoethyl) phosphate·3HCl (SKF 7997-A_3)

FIG. 1. The structure of SKF 525-A and some of its analogs.

The inhibition of multiplication by SKF 525-A or SKF 3301-A was annulled only by oleic acid (Table 2). The following lipids equimolar with oleic acid were inactive: mevalonic acid lactone, farnesol, geraniol, squalene, ergosterol, lanosterol, cholesterol, stigmasterol, and β-sitosterol; and lauric, myristic, palmitic, and stearic acids. While no lipid annulled the inhibition by SKF 2314 or

TABLE 2. EFFECT OF OLEIC ACID ON INHIBITION OF MULTIPLICATION BY SKF 525-A AND ITS ANALOGS

		% of Normal multiplication		
		Conc. of oleic acid (mM)		
SKF Compound	Conc. (mM)	0	0·04	0·35
	0	100	104	111
525-A	0·03	99	99	110
	0·05	22	19	97
	0·08	0	0	92
3301-A	0·06	104	104	109
	0·12	93	104	109
	0·17	16	41	90
	0·23	0	0	16
2314	0·04	93	98	110
	0·08	0	0	0
16467-A	0·56	92	90	90
	0·70	70	65	65
	0·84	49	10	20
	0·98	11	0	0
	1·12	0	0	0

TABLE 3. EFFECT OF L-CYSTINE ON INHIBITION OF MULTIPLICATION BY SKF 2314

		% of Normal multiplication					
		Conc. of L-cystine (mM)					
Compound	Conc. (mM)	0	0·04	0·12	0·42	0·84	1·26
SKF 2314	0	100	98	98	98	98	97
	0·04	97	95	97	95	93	96
	0·06	93	96	97	95	95	96
	0·08	0	0	0	37	83	93
	0·12	0	0	0	0	74	92

SKF 16467-A, the SKF 2314 inhibition was annulled by complete supplement—a mixture of water-soluble organic compounds including amino acids, B-vitamins, purines, and pyrimidines. The active compound was L-cystine (Table 3); L-cysteine was inactive.

DISCUSSION

The diphenylpropylacetate moiety is necessary for the inhibition of *O. danica* multiplication (Fig. 1). Compounds lacking this group but resembling the diethylaminoethanol portion of SKF 525-A were inactive. The SKF compounds described here had the same pattern of inhibition on aerobic respiration of *O. danica* as they had on multiplication (unpublished results).

The inhibition induced by SKF 525-A and SKF 3301-A was annulled by oleic acid. SKF 2314 and 16467-A inhibitions were not annulled by lipids; SKF 2314 was annulled by L-cystine. SKF 16467-A inhibition was not annulled by any of the lipids or water-soluble compounds that annulled inhibitions by other SKF compounds. That SKF 525-A acts on a different metabolic site than its acid analog (SKF 2314) may be seen by the results described here and in other biological systems: O-demethylation of O-nitroanisole is blocked by SKF 525-A but not by SKF 2314, and SKF 525-A inhibits drug metabolism *in vivo* whereas SKF 2314 does not.[5] The specific site(s) of action of these compounds remains to be determined.

Haskins Laboratories, New York, and
Biology Department, Queens College,
City University of New York,
Flushing, N.Y., U.S.A.

S. AARONSON

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