ANTIFUNGAL ACTIVITY OF CROWN ETHERS

KOJI YAGI, VERONICA GARCIA, MA. E. RIVAS, JAVIER SALAS,
ANDRES CAMARGO, TAKEO TABATA*
Departamento de Química, *Departamento de Química Agricola
ICET, Universidad autónoma de Guadalajara, Apdo Postal 1-440
Lomas del Valle, Guadalajara, Jalisco, México, 44,100

ABSTRACT. Some crown ethers were found to show significant antifungal activity against some wood-decay fungi, phytopathogenic fungi and eumycetes , *Trichophytons* for dermatomycosis. Their toxicity was evaluated by the paper disc method as well as by determining the values of ED_{50}, i.e., the concentration which inhibits the mycelium growth by 50 %. The fungi examined are *Tyromyces palustris, Picnoporous coccineus, Coriolous versicolor, Pyricularia filamentosa, Fusarium sp., Trichophyton rubrum, Trichophyton sp.*, etc. Among the 26 crown ethers tested, 3,5-di-t-butylbenzo-15-crown-5 showed relatively high activity, the highest ED_{50} value of which being 8 μM or 3 ppm. Other alkylbenzocrown ethers, dicyclohexyl crown ethers and Kryptofix 22DD also showed considerable activity. On the other hand, unsubstituted crown ethers, benzocrown ethers with a polar substituent, Kryptofix 222B and Kryptofix 221 were inactive.

Introduction

Macrocyclic polyethers, commonly called crown ethers, resemble natural ionophores like valinomycin, the nactins etc., in a macrocyclic structure with a hydrophobic outersphere and a hydrophilic cavity capable of forming complexes with cations with high selectivity. (1). Although innumerable papers have been published concerning their chemical properties, not much work has been done regarding their biological properties. Oral toxicity in dogs and in mice was reported by Takayama et al. (2) and by Hendrixson et al. (3), respectively. Wung-Wai Tso et al. studied the toxicity of some crown ethers against *Escherichia coli*. (4,5,6). Kato et al. found that long chain alkylcrown ethers like decyl-18-crown-6 showed strong antimicrobial activity against some Gram-positive bacteria, mold and yeast. (7,8). More recently, some crown ethers are reported to show anticoccidal activity against *Eimeria tenella in vitro* but not *in vivo*. (9). An application as feed preservatives has also been claimed in a patent. (10).

In this paper we describe our finding that some alkylbenzocrown ethers show significant antifungal activity against some wood-decay fungi, phytopathogenic fungi, and *Trichophytons* for dermatomycosis.

Journal of Inclusion Phenomena **2**, 179–184. 0167-7861/84.15.

Preliminary results of this study was reported previously. (11)

Materials and Method

Alkylbenzocrown ethers were prepared according to Pedersen's method (12) and were characterized by melting point, IR and NMR spectra, e.g., 8 was prepared by reacting 3,5-di-t-butylcatechol and dichloride of tetraethylene glycol, mp, 98-100 °C, NMR ($CDCl_3$) δ 1,30, 1.40(18H,2s,2-t-butyl, 3.65-4.40 (16H,m,-CH_2CH_2O-), 6.80-7.00(2H,m,arom.) See reference (11) for the details. Commercial crown ethers were used without further purification.

All the fungi used in this study are those preserved in our laboratory. *Trichophytons* were examined on Sabouraud's culture medium and all the others on the potato-dextrose agar culture medium. For the qualitative test by the paper disc method, a few percent solution of crown ether in THF or in DMF was used. The ED_{50} values were determined as follows: 1 ml of crown ether solution in THF or in DMF was dispersed in 100 ml of appropriate culture medium at about 50°C and the solution was poured into four petri dishes. Discs of fungal mycelium growing on the same culture medium were placed in the center of the plates with different crown ether concentration. The plates were then incubated at about 25°C for 4-14 days. The fungal growth was determined by measuring the mycelium length of the control plate devoid of crown ether (lo) and that of the test plate (l). The inhibitory activity was defined as (lo - l)/lo x 100 (%). The plates were made in quadruplicate in all cases, the reproducibility of the data being generally good. The ED_{50} values, i.e., the concentration which inhibits the mycelium growth by 50 %, was obtained by plotting the Probit of the averaged inhibitory activity and the logarithm of the crown concentration.

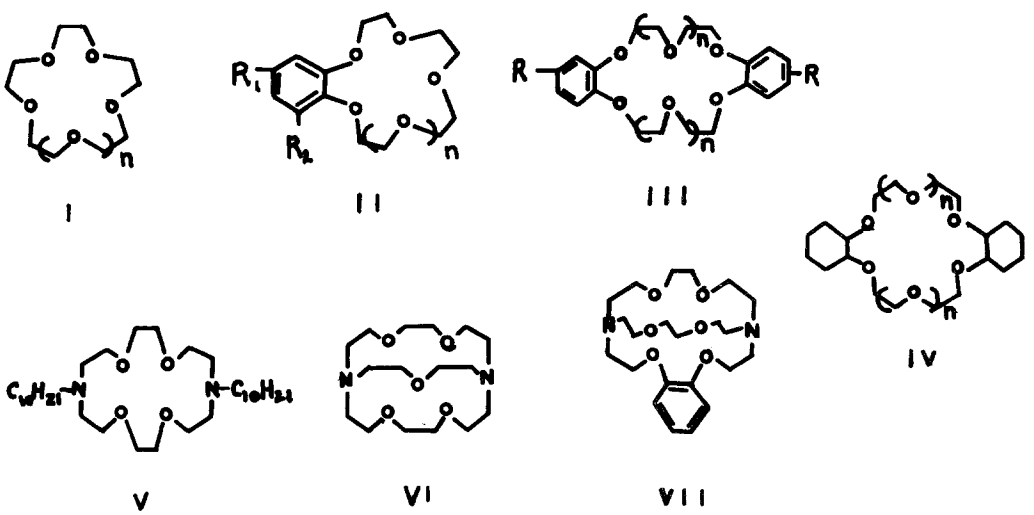

Figure 1. The structures of crown ethers used in this study.

Results and Discussions

The structures of crown ethers used in this study are shown in Figure 1. In table 1 are shown the results of the qualitative test by the paper disc method. Some alkylbenzocrown ethers (type II and III) and dicyclohexylcrown ethers (type IV) showed certain activity. On the other hand, unsubstituted crown ethers (type I) and benzocrown ethers with a polar substituent were inactive. Kryptofix 221 (VI) and Kryptofix 222B, which are highly selective to sodium and potassium, respectively, proved to be inactive, whereas Kryptofix 22DD, which has long alkyl chains, showed some activity.

Table 1. The results of qualitative test against *Trichophytons*.

No	Structure[a]			Activity[b]		No	Structure			Activity[b]	
	Type,	n,	R_1	A[c]	B[c]		Type,	n,	R_1 or R	A[c]	B[c]
1[d]	I	0		-	-	14	II	2	H	+-	+-
2[d]	I	1		-	-	15	II	2	methyl	+	+
3[d]	I	2		-	-	16	II	2	t-butyl	++	++
4	II	0	H	-	+	17[e]	II	2	nitro	-	-
5	II	1	H	+	+-	18[e]	II	2	X[i]	-	-
6	II	1	methyl	+	+-	19	III	1	H	-	-
7	II	1	t-butyl	+++	+++	20[k]	III	1	t-butyl	++	++
8	II	1	t-butyl	j	j	21[g]	III	2	H	-	-
9[e]	II	1	nitro	-	-	22[g]	IV	1		++	++
10[e]	II	1	amino	-	-	23[g]	IV	2		+	+
11[f]	II	1	CH_3CO	-	-	24[h]	V			+	++
12[f]	II	1	$CH_3CH(OH)$	-	-	25[h]	VI			-	-
13[e]	II	1	X[i]	-	-	26[h]	VII			-	-

a, R_2=t-butyl for 8 and H for all others. b, The zone of inhibition was larger than 7 mm (+++); from 4 mm to 7 mm (++); less than 4 mm (+); questionable inhibition (+-); no detectable inhibition (-). c, A: *Trichophyton sp.*, B: *Trichophyton rubrum*. d,g,h, Purchased from Aldrich Chemical Co., Nippon Soda Co., Kanto Chemical Co., respectively. e,f, For the synthesis see reference 13 and 14, respectively. i, X: $CH_2=C(CH_3)CONH$. j, The test was not done for this compound. k, See text.

In table 2 is shown the inhibitory activity of some crown ethers against three fungi. Some of them obviously suppressed the growth of the fungal mycelium. In the series of benzo-15-crown-5 derivatives (5, 6, 7) and benzo-18-crown-6 derivatives (14, 15, 16), a tendency is observed that the inhibitory activity increases with the increase of alkyl group, i.e., t-butyl > methyl > H. 20 showed relatively high activity. Dicyclohexyl-18-crown-6 (22) showed higher activity than dicyclohexyl-24-crown-8 (23) and dibenzo-24-crown-8 (21). Although there is observed some selectivity in their inhibitory activity, relatively high activities were observed for benzocrown ethers with t-butyl group (7, 16, 20) and dicy-

Table 2. Inhibitory activity of crown ethers against some fungi.

Crown Ether[b]	Inhibitory activity (%)[a]		
	Trichophyton rubrum	*Picnoporous coccineus*	*Pellicularia filamentosa*
5 (B15C5)	7	1	-
6 (MB15C5)	14	6	2
7 (tBuB15C5)	64	54	78
14 (B18C6)	4	17	34
15 (MB18C6)	18	28	53
16 (tBuB18C6)	38	65	61
20 (see text)	61	84	66
21 (DB24C8)	14	24	35
22 (DCH18C6)	57	56	89
23 (DCH24C8)	10	22	28
24 (KPF22DD)	63	80	47

a, See text for the definition. The concentration of crown ether was 144 μM in all cases. b, See table 1 for the structure.

Table 3. Toxicity of crown ethers against some wood-decay fungi.

Crown Ether	ED_{50} (μM) for		
	Coriolous versicolor	*Picnoporous coccineus*	*Tyromyces palustris*
7 (tBuB15C5)	105	140	78
8 (DtBuB15C5)	30	17	20
16 (tBuB18C6)	110	84	76
22 (DCH18C6)	53	120	58
24 (KPF22DD)	42	26	140

Table 4. Toxicity of crown ether against some phytopathogenic fungi.

Crown Ether	ED_{50} (μM) for		
	Fusarium sp.	*Pellicularia filamentosa*	*Pyricularia oryzae*
7 (tBuB15C5)	31	51	-
8 (DtBuB15C5)	8.4	23	39
16 (tBuB18C6)	106	120	-
22 (DCH18C6)	-	38	-

clohexyl-18-crown-6 (22) and Kryptofix 22DD (24).

To evaluate the toxicity quantitatively, the ED_{50} values were determined by the method described in the preceding section. The results for

some wood-decay fungi and phytopathogenic fungi are shown in table 3 and 4, respectively. As shown in these tables, di-t-butylbenzo-15-crown-5 (8) showed notable antifungal activity, the highest ED_{50} values of which being 8.4 μM or 3.2 ppm. t-Butylbenzo-15-crown-5 (7), dicyclohexyl-18-crown-6 (22) and Kryptofix 22DD (24) also showed considerable activity in some cases, though their activities were less than 8. 20 is a product obtained by reacting 4-t-butylcatechol and bischloroethyl ether and is supposedly di-t-butyldibenzo-18-crown-6. It showed ED_{50} values comparable to those of 8 against wood-decay fungi and phytopathogenic fungi shown in table 3 and 4, however, since its melting point is much lower than that reported in the literature (12), the data are not included in these tables. Details will be published eleswhere when its structure is well confirmed.

ED_{50} values of 7 and 8 against *Trichophyton sp.* were 67 μM and 16 μM, respectively. 7 also showed activity against *Trichophyton rubrum,* and *Trichophyton mentagrophytes,* the ED_{50} values of which being 73 μM for the former one, however, for the latter one, we could not determine accurately the ED_{50} since the fungal mycelium sometimes did not grow uniformly but formed an irregular shape, in addition, the mycelium growth was very late.

Although the toxicity of crown ether itself and the antifungal activity *in vivo* have to be examined carefully before thinking about their application, it is interesting to note that such crown ethers, which show interesting chemical properties, also show biological activity. The mechanism of their activity is not known at all, however, one may possibly imagine that they act like natural ionophores. Since the results obtained here indicate that the antifungal activity increases with the increase in the lipophilic property, we are trying the synthesis of more lipophilic crown ethers. We are also interested in elucidating the relation between the antifungal activity and the binding properties as well as the lipophilic properties of the crown ethers.

Acknowledgements

The authors are grateful to CONACYT, Consejo Nacional de Ciencia y Tecnologia, and Universidad Autónoma de Guadalajara for their financial support to present this work at the international symposium on III MIP and II CD.

References

1) Yu.A. Orchinnikov, V.T. Ivanov and A.M. Shkrob, Membrane-Active Complexones, Elsevier (1974)
2) Kozo Takayama, Shigeki Hasegawa, Sumiko Sasagawa, Naoki Nambu and Tsuneji Nagai, Chem. Pharm. Bull., 25, 3125 (1977)
3) Richard R. Hendrixson, Mark P.Mack, Richard A. Palmer, Athos Ottolenghi and Robert G. Ghirardelli, Toxicol. Appl. Pharmcol., 44, 263 (1978)
4) Wung-Wai Tso and Wai-Ping Fung and Man-Yin Wong Tso, J. Inorg.

Biochem., 14, 237 (1981)
5) Wung-Wai Tso and Wai-Ping Fung, Inorg. Chim. Acta.,55, 129 (1981)
6) Wung-Wai Tso and Wai-Ping Fung, Microbios. Lett., 19, 145 (1982)
7) Nobuyuki Kato, Isao Ikeda, Mitsuo Okahara and Isao Shibasaki, J. Antibact. Antifung. Agents, 8, 532 (1980)
8) Nobuyuki Kato, Isao Ikeda, Mitsuo Okahara and Isao Shibasaki, J. Antibact. Antifung. Agents, 8, 415 (1980)
9) George R. Brown and Alan J. Foubister, J. Med. Chem., 26, 590 (1983)
10) Ajinomoto Co. Inc., Japan Kokai Tokkyo Koho, 82,209,220; Chem. Abst. 98-124505
11) Koji Yagi, Maria Cristina Sanchez and Takeo Tabata, Rev. Mex. Cien. Farmac., 14, 24 (1984)
12) C.J. Pedersen, J. Am. Chem. Soc., 89, 7017 (1967)
13) Koji Yagi, Jorge A. Ruiz, Maria Cristina Sanchez, Makromol. Chem. Rapid Commun., 1, 263 (1980)
14) S. Kopolow, T.E. Hogen Esch and J. Smid, Macromolecules, 6, 133 (1973)