has two invariant distances to some two used ligand points, the position of the unused one is restricted to lie on a circle in space. If there are four or more site points which also lie on that circle (as indicated by their having the same corresponding distances to the two site points involved in contacts with the two used ligand reference points), then the unused point in question must be in contact with one of these four site points. Take the new contact to be with the energetically most favorable unused site point of the four. Of course, the choice of 4 as the number of site points to completely occupy a circle in space is rather arbitrary, and increasing the number would amount to making the search more detailed. The main use of this sort of forced contact is to require a part of the ligand that can rotate to either find an energetically favorable orientation in the site or be excluded altogether in the case that all four site points are repulsive. (8) The last sort of forced contact to be considered is the single-point variety, where making one contact constrains an unused ligand point with invariant distance to the used one, to lie on a spherical shell in space. If there are six site points that also lie on this shell, then the unused ligand point is taken to be in contact with the most energetically favorable unused one. This is certainly the situation in a concave site "pocket", and once again the choice of six is arbitrary (except that it should clearly be greater than the number of site points necessary to force a double-point contact). (9) As indicated above, triple-point forced contacts are the most specific and are tried first until no more can be made. Only then are double-point contacts attempted. If one is formed, then perhaps triple-point contacts can again be deduced, so that must be tried again exhaustively. Only when no more triple- or double-point contacts can be formed are the single-point forced contacts tried. Once again, success results in trying triple points again. When at last no contacts of any variety can be forced, the proposed contact combination is considered to be complete. (10) The energy of the (possibly revised) contact combination is evaluated simply by summing the energetic contributions of each contact. The contribution is taken to be the given interaction energy table entry for the corresponding ligand point type and site point type. Unused ligand or site points contribute zero to the sum. The calculated mode of binding is the contact combination that gives the minimal calculated binding energy.

Fortran programs exist for the above algorithms. Since this work is still in its early stages, these programs are probably difficult for the uninitiated to use. We anticipate improving the methodology, so that our approach may be easily employed by others, but in the meantime the author may be contacted about possible applications.

References and Notes

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Notes

Synthesis of Benzo-15-crown-5 Polyethers, Anticoccidial Ionophore Analogues

George R. Brown* and Alan J. Foubister

Imperical Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, England.

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Synthesis of eight benzo-15-crown-5 derivatives I (R = H, CO_2Me, CO_2H, Me; R_1 = H, CO_2H, CO_2Me, CHO, $CH{=}CHCO_2H$, $CH_2CH_2CO_2H$) designed as rigid cyclic analogues of the anticoccidial ionophores is described. No anticoccidial activity was observed in chickens, but moderate activity in tissue culture was found for I (R = Me, R_1 = H; R = R_1 = H) and dibenzo-18-crown-6.

The synthesis of acidic derivatives of benzo-15-crown-5 I designed as rigid cyclic analogues of the anticoccidial ionophores is described. No anticoccidial activity was observed in chickens but moderate activity in tissue culture

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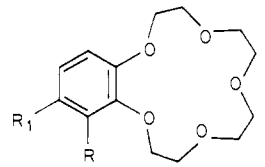
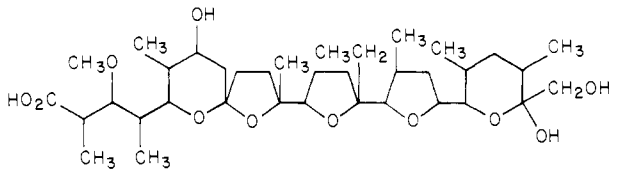


I

was found for 3′-methylbenzo-15-crown-5.

Gardner et al.[1] have described the synthesis of acyclic carboxyl-containing polyethers as analogues of the anticoccidial ionophore monensin (1). None of these com-



1

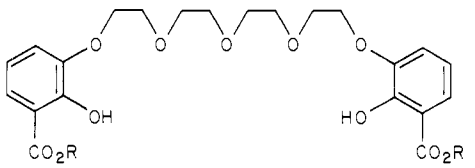
pounds was active, and a possible explanation is that they lack the rigidity imposed on the monensin molecule by heavy alkyl group substitution and a spiro center. In addition, 1 is a linear molecule which is held in a cyclic form in the solid state[2] and in solution[3] by intramolecular "head to tail" hydrogen bonds. For this reason, the cyclic crown polyethers (Table I) resemble monensin because they are rigid and cyclic. They are able to transport alkali metal cations across biological membranes, and different macrocyclic ring sizes show selectivity toward specific metal cations.[4]

The benzo-15-crown-5 ring system was chosen as the basis for this work because, like 1, it is selective for sodium cations. Anticoccidial ionophores are monocarboxylic acids, and thus acidic crown polyethers are appropriate target molecules. The counteranion of a cation complexed with a crown ether has been shown by X-ray study[5] to be some distance from the cation; therefore, the acidic aromatic carboxyl groups maybe able to act as counterions to sodium ions complexed in the polyether ring.

The compounds synthesized have been screened against the caecal coccidium *Eimeria tenella* in chickens and in chicken kidney tissue culture.[6]

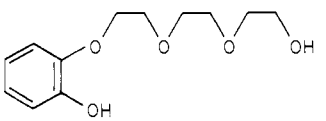
Chemistry. The acid 3 was prepared via alkylation of methyl 3,4-dihydroxybenzoate with the ditosyl derivative of tetraethylene glycol in THF in the presence of sodium hydride to give the ester 2. Hydrolysis of 2 with sodium hydroxide afforded the acid 3. This route gave a higher yield in fewer stages than the literature method from veratrole.[7] Similar alkylation of protocatechualdehyde in the presence of sodium hydroxide gave the aldehyde, but a superior yield was obtained in 1-butanol using 1,11-dichloro-3,6,9-trioxaundecane as the alkylating agent. Knoevenagel condensation of 4 with malonic acid and catalytic reduction readily gave 5 and 6.

No 3-substituted benzo-15-crown-5 derivatives are reported in the literature, and syntheses proceeded in poor yield. Thus, alkylation of methyl 2,3-dihydroxybenzoate under the same conditions as for 2 gave 7, together with a substantial amount of the bis(monoalkylation) product 10. The diester 10 hydrolyzed readily to the diacid 11, but the crown ester 7 on hydrolysis with sodium hydroxide solution gave, on acidification with dilute hydrochloric acid, a sodium complex 13, including chloride anion as the counterion. Filtration of this complex through an ion-exchange column yielded the free acid 8. The structure of 10 was based on the absence of an aromatic proton signal corresponding to a proton ortho to a phenolic hydroxyl in the ^{1}H NMR spectrum and the ester absorption



10, R = CH_3
11, R = H

at 1670 cm^{-1} in the IR spectrum, suggesting hydrogen bonding of the ester group. The formation of the bisether 10 was probably due to lowering of the reactivity of the hydroxyl group next to the carboxyl group by intramolecular hydrogen bonding. This problem could be eliminated if the benzo-15-crown-5 ether of 3-methylcatechol was prepared and the methyl group subsequently oxidized to carboxyl. In practice, 9 was difficult to prepare and only a trace was obtained using the alkylation conditions for 2. Using sodium hydroxide in butanol, an 8% yield of 9 was obtained. Oxidation of 9 to 8 with hot aqueous potassium permanganate solution, however, did not proceed. An alternative approach to the synthesis of acidic benzo-15-crown-5 derivatives by lithiation of benzo-15-crown-5 with butyllithium, followed by carboxylation with carbon dioxide, has been explored using Gilman's method.[8] No acidic products were present in the reaction, and the dealkylation product 12 was isolated. The structure assigned for 12 was based on ^{1}H NMR and mass spectra.

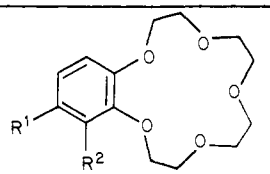


12

Biological Results and Discussion. None of the compounds described was active against *Eimeria tenella* in chickens at a dose of 1000 ppm in the feed, whereas 1 was markedly active at 120 ppm in the same test. None of the new compounds was active against coccidia in tissue culture, except 9, which was found active down to 9 ppm. This moderate activity of a crown polyether suggested the exploration of the relationship between ring size, oxygen basicity, and anticoccidial activity in other crown ethers. The acyclic compounds 10–12 were inactive. Activity of 9 ppm was also found in the tissue culture assay for benzo-15-crown-5 and dibenzo-18-crown-6. The more basic and less lipophilic nonaromatic crown polyethers 15-crown-5 and 18-crown-6, which form more stable complexes with the same alkali metal cations, were inactive. The failure of the acidic polyethers 3, 5, 6, and 8 to show useful anticoccidial activity may be due to the low lipophilic character of these molecules or to the lack of shielding of the carboxyl groups, which is shielded in the cyclic form of 1. The sodium salts of the acids 3, 5, and 6 did not behave as lipophilic materials, since they were not extracted from aqueous solutions by water-immiscible organic solvents in contrast to the sodium salt of 1. The fact that acidification of the sodium salt of 8 with an excess of hydrochloric acid afforded a sodium complex 13 demonstrated the ability of the molecule 8 to behave as an ionophore. The presence of chloride anion in the complex 13, however, showed that the carboxyl group in 8 was not involved in complexation and suggested that planar aromatic carboxyl groups may be unable to act as counteranions to a complexed sodium cation.

Careful examination of 9 in tissue culture showed it to be active against the second generation schizont stage of the parasite life cycle, whereas 1 was active against the first stage. This difference in the anticoccidial activity may only

Table I. Benzo-15-crown-5 Derivatives



no.	R^1	R^2	yield, %	mp, °C	formula
2	CO_2Me	H	14	81-83	$C_{16}H_{22}O_7$
3	CO_2H	H	83	184-186	$C_{15}H_{20}O_7$
4	CHO	H	29	70-72	$C_{15}H_{20}O_6$
5	$CH=CHCO_2H$	H	21	192-194	$C_{17}H_{22}O_7$
6	$CH_2CH_2CO_2H$	H	50	96-98	$C_{17}H_{24}O_7$
7	H	CO_2Me	11	67-68	$C_{16}H_{22}O_7$
8	H	CO_2H	45	87-89	$C_{15}H_{20}O_7$
9	H	Me	8	37-38	$C_{15}H_{22}O_5$

be a reflection of the relative degree of activity of 9 and 1 and does not necessarily indicate that 9 is acting by a mechanism which does not involve ion transport. Delaying the dosing of monensin to the second schizont stage of the life cycle also kills the coccidial parasite in tissue culture and in vivo in chickens. In addition, the first and second schizont stages are very similar and occur in identical cells in the tissue culture assay.

Experimental Section

Melting points are uncorrected. IR and NMR were determined on a Perkin-Elmer 157 and a Varian HA 100 spectrometer, respectively. Spectral data were consistent with the assigned structures and were supported by MS fragmentations from a Hitachi RMU-6E instrument. Where analyses are indicated only by symbols, elementary analyses are within ±0.4% of the theoretical values.

4'-Carbomethoxybenzo-15-crown-5 (2). Ditosyltetraethylene glycol[9] (8.50 g, 16 mmol) in THF (100 mL) and 80% NaH (0.50 g, 16 mmol) were added simultaneously under nitrogen during 6 h to a stirred solution of methyl 3,4-dihydroxybenzoate (2.88 g, 16.6 mmol) and 80% NaH (0.50 g, 16.6 mmol) in THF (200 mL) at 35–40 °C. Stirring at 35–40 °C was continued for 18 h. The mixture was cooled, water (0.5 mL) was added, and the mixture was filtered. The THF was evaporated to give an oil, which was purified by column chromatography on alumina ($CHCl_3$) and crystallized (Et_2O) to give 2 (0.70 g, 14.0%), mp 81–83 °C, lit.[7] 82 °C. Anal. ($C_{16}H_{22}O_7$) C, H.

4'-Formylbenzo-15-crown-5 (4). Protocatechualdehyde (4.1 g, 30 mmol) and NaOH (2.5 g, 62.5 mmol) were added to water (4 mL) and *n*-BuOH (150 mL), and the mixture was heated to reflux under nitrogen. 1,11-Dichloro-3,6,9-trioxaundecane[10] (6.9 g, 30 mmol) in *n*-BuOH (50 mL) was added with stirring during 30 min, and heating at reflux continued for 18 h. The cooled mixture was acidified with HCl and filtered. *n*-BuOH was evaporated from the filtrate, and the resulting oil was extracted with hot toluene. The toluene was evaporated and the oil filtered through alumina in $CHCl_3$. Evaporation and crystallization of $CHCl_3$ eluates from toluene–*n*-hexane gave 4 (2.4 g, 29%), mp 70–72 °C. Anal. ($C_{15}H_{20}O_6$) C, H.

4'-(3-Acrylyl)benzo-15-crown-5 (5). Malonic acid (1.5 g, 15 mmol) and 4 (1.0 g; 3.3 mmol) were heated for 3 h at 100 °C in pyridine (3 mL) and piperidine (5 drops). The solvent was evaporated and the residue was crystallized from EtOH to give 5 (250 mg, 21%), mp 192–194 °C. Anal. ($C_{17}H_{22}O_7$) C, H.

4'-(2-Carboxyethyl)benzo-15-crown-5 (6). The acid 5 (200 mg, 0.575 mmol) in EtOH (20 mL) and HOAc (10 mL) was hydrogenated at 1 atm over 5% Pd/C (50 mg) for 30 min. The catalyst was filtered and the solvents were evaporated. Crystallization of the residue from toluene gave 6 (100 mg, 50%), mp 96–98 °C. Anal. ($C_{17}H_{24}O_7$) C, H.

3'-Carbomethoxybenzo-15-crown-5 (7) and Ethylene Glycol Bis[(2-hydroxy-3-carbomethoxyphenoxy)ethyl] Ether (10). Methyl 2,3-dihydroxybenzoate (1.68, 0.01 mol), following the method for 2, gave a mixture which was purified by column chromatography on deactivated silica gel (EtOAc) and crystallized from Et_2O to give 10 (350 mg, 13%), mp 72–73 °C. Anal. ($C_{24}H_{30}O_{11}$) C, H. Further elution with EtOAc and crystallization from petroleum ether, 60–80 °C, gave 7 (350 mg, 11%), mp 67–68 °C. Anal. ($C_{16}H_{22}O_7$) C, H.

Ethylene Glycol Bis[(2-hydroxy-3-carboxyphenoxy)ethyl] Ether (11). The ester 10 (334 mg, 0.67 mmol) was hydrolyzed with NaOH (500 mg, 5 mmol) in water (8 mL) in the same way as 3. Crystallization from EtOH–H_2O gave 1 (238 mg, 74%), mp 89–91 °C. Anal. ($C_{22}H_{26}O_{11}$) C, H.

3-Carboxybenzo-15-crown-5 (8). The ester 7 (266 mg, 0.8 mmol) was hydrolyzed with NaOH (100 mg, 2.5 mmol) as for 3, and the solution obtained after acidification was evaporated under reduced pressure. The residue was treated with EtOH and filtered to give 13 (320 mg, 74%), mp 58–62 °C. Anal. ($C_{15}H_{20}O_7$·NaCl) C, H. Filtration of 13 in water through Bio-Rad AG50W-X2 resin (H^+ form) gave 8 (140 mg, 56%), mp 87–89 °C. Anal. ($C_{15}H_{20}O_7$) C, H.

Tetraethylene Glycol 2-Hydroxyphenyl Ether (12). *n*-BuLi (5.2 mL, 1.6 M in *n*-hexane, 8 mmol) was added to benzo-15-crown-5 (1.1 g, 4 mmol) in dry THF (40 mL) under N_2 over 5 min. After 5 min, the reaction was poured onto solid CO_2 under ether, and the CO_2 was allowed to evaporate. The oily residue was dissolved in dilute HCl and extracted with EtOAc. The aqueous phase was evaporated and the residue was purified by preparative TLC on silica gel using EtOAc as eluant to give a colorless oil 12 (190 mg, 19%): NMR ($CDCl_3$) δ 6.83 (s, 4 H, arom), 5.10 (m, 2 H, OH), 4.00 (q, 2 H, CH_2OH), 3.73 (q, 2 H, arom OCH_2), 3.60 (s, 8 H, CH_2O $(CH_2)_2OCH_2$]; MS *m/e* 242 (M^+).

Anticoccidial Activity. Groups of eight cock chickens, 10 days of age, were fed a diet medicated with the compounds under test at a concentration of 1000 ppm and inoculated with a lethal dose of *E. tenella* oocysts. Anticoccidial activity was assessed on the basis of weight gain, mortality, and faecal blood over a period of 7 days. Cultures of kidney cells from 3-week-old chickens were inoculated with sporozoites of *E. tenella* and exposed to threefold serial dilutions of the compound under test. Anticoccidial activity was assessed 4 days later on the basis of the presence or absence of second-generation schizonts of the parasite. The initial dose used in the tissue culture test was 81 ppm. Those compounds described as inactive failed to prevent the formation of second generation schizonts at this dose level. Methods are described in full by Ryley and Wilson.[6]

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