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Bis-Cyclohexyl-Crown-Ethers as Allosteric Carriers

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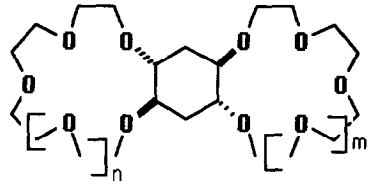
Abstract: *Several bis-cyclohexyl-crown-ethers have been synthesized and used as carriers for alkaline cations. These compounds should all show negative allosteric cooperativity , but only 1 exhibits an odd cation transport behavior across the liquid organic membranes.*

The activity of allosteric enzymes is regulated by conformational changes induced by the reversible binding of a number of agents, and several models of allosteric cooperativity have been described[1]. In devising crown ethers with positive allosteric cooperativity[2] and using them as carriers in transport experiments, we recently prepared a crown ether **1**[3] which seems to exhibit negative allosteric cooperativity. Compound **1**appears to be useful as a model of the plasma membrane Na^+-K^+ATPase, which actively pumps Na^+ out and K^+ into the cell, to regulate cytoplasmic ion concentrations.

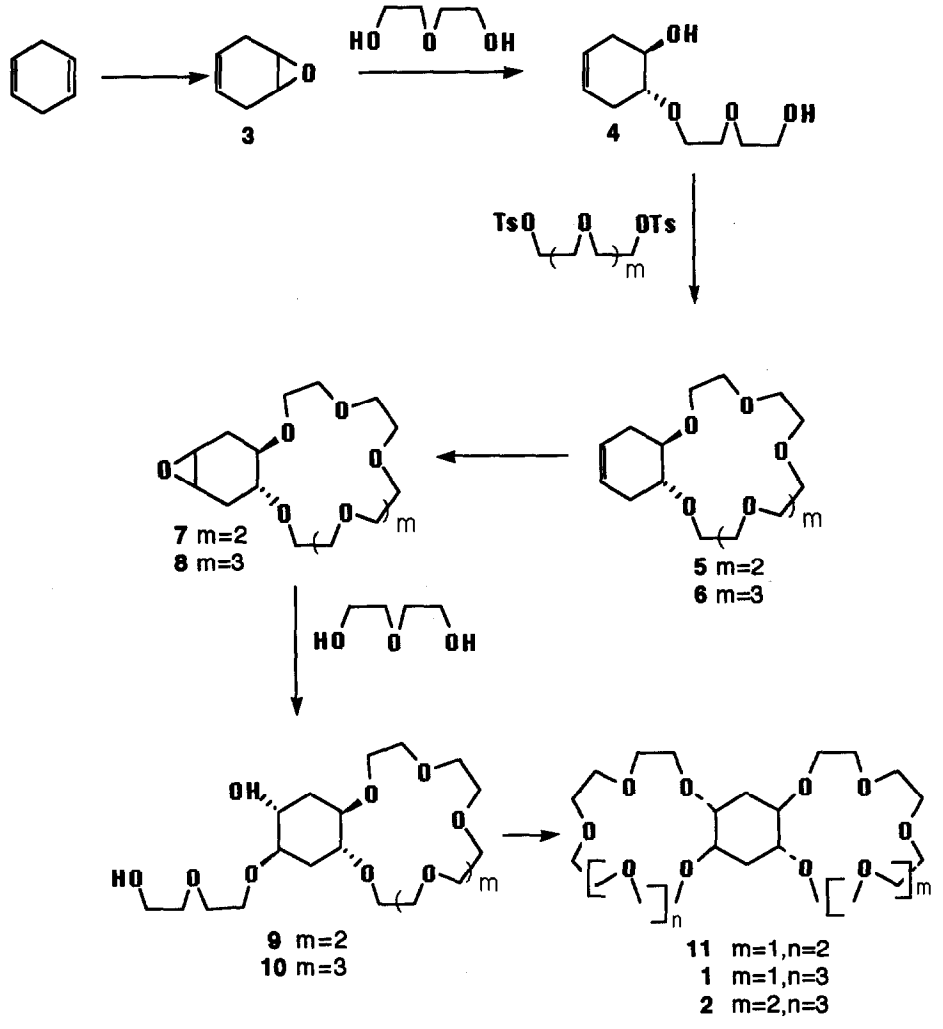


1 n=1, m=3
2 n=1, m=2

Transport experiments carried out with **1** showed that this system is able to transport twice as much Na^+ and K^+ than the two corresponding monocyclic compounds combinated. This odd behavior could be explained as a consequence of the negative allosteric cooperativity as in this kind of system the single subunit crown ether complex formation forces the two oxygens to remain in the diequatorial conformation. This conformation is transmitted through the cyclohexane to the second crown ether subunit; consequently this latter subunit has its two oxygens in the diaxial conformation and the complex formation is hindered.

With the purpose of performing an in-depth study of negative allosteric cooperativity and its influence on cation transport, we prepared compound **2** by using a similar synthetic route (Scheme 1). Synthesis of compounds **1** and **2** was carried out from 1,4-cyclohexadiene which was converted

into its monoepoxide by reaction with hydrogen peroxide and ethyl chloroformate[4]. The monoepoxide was opened with diethylene glycol to the trans compound **4**[5], from which the first ethereal cavity was constructed by condensation with the appropriate glycol ditosylate[6]. Repetition of the sequence leads to trans diaxial opening of the epoxide to the second diol necessary for the construction of the second cavity[7].

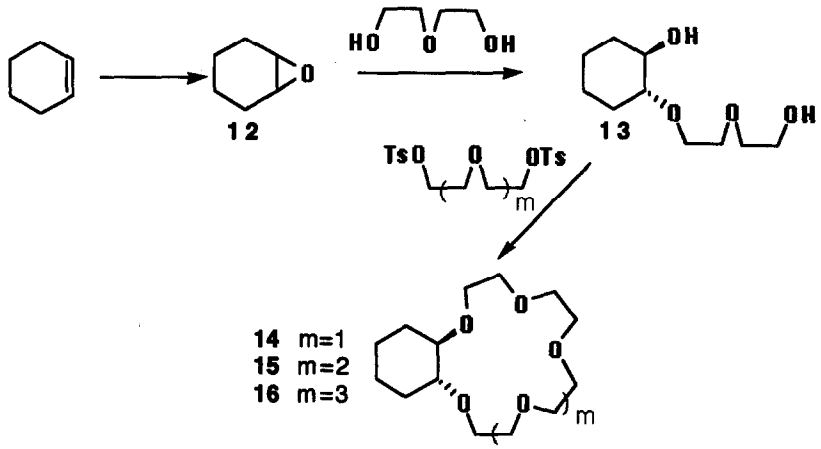


Scheme 1

The stereochemistry trans-transoid-trans of compounds **1**, **2** and **11** was determined by ^1H NMR using Eu (hfc)$_3$ because they are chiral compounds. In these experiments double signals were observed not only for the crown-ether methylenes, but also for for the cyclohexane hydrogens.

In order to obtain similar compounds without allosteric cooperativity to be used in control experiments we have prepared cyclohexyl-15-crown-5 (**14**), cyclohexyl-18-crown-6 (**15**) and

cyclohexyl-21-crown-7 (**16**). These compounds have been synthesized in a way similar to cyclohexane (Scheme 2).



Scheme 2

The epoxide **8** has been studied by NMR to establish its conformation. A simulation of this compound by the MMX program and drawn through Chem 3D is shown in Fig 1. Theoretical results confirm the spectroscopic data that show a strongly predominant boat conformation in the cyclohexane ring of compound **8**. Such a conformation is not only supported by the ^1H NMR spectra, but also by the result of the NOEDIF experiments. As the H^1 NMR data show, this compound, is able to complex $Hg(SCN)_2$; the structure determination of this complex is in progress.

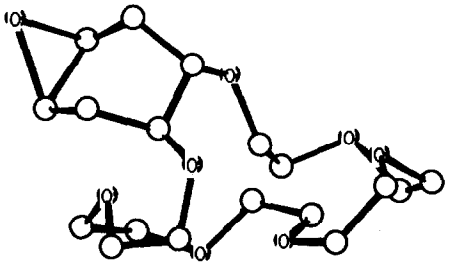


Fig.1. Simulated structure of epoxicompound **8**

In compound **2** the difference in the hole size of both crown ether subunits is less than in **1**, In this way we could study not only the negative allosteric cooperativity but also the influence of the crown ether moiety sizes as well. To carry out this study we determined the association constants. These values in $CHCl_3$ at 24 ° C were obtained by the extraction method described by Cram[8].

Table 1 shows the association constants, while the Experimental Section provides the details of the method used.

Table 1. Association Constants Determined by the UV Method.

Carrier	K	Na
CH-5 (**14**)	2.52 10^3	7.81 10^3
CH-6 (**15**)	9.89 10^3	5.17 10^3
CH-7 (**16**)	6.49 10^3	2.45 10^3
5-CH-7 (**1**)	4.49 10^2	1.21 10^2
6-CH-7 (**2**)	1.77 10^3	6.50 10^2

Predictably, crown ethers with 5 oxygens complex Na^+ better than K^+, while crown ethers with 6 oxygens show the opposite preference. In compound **16**, a crown ether with 7 oxygens and a bigger hole size, the association constants with both cations are smaller. Bis-cyclic compounds **1** and **2** have less affinity for alkali cations than monocyclic structures and consequently the association constants are smaller.

On the other hand, conformational analysis according to the rigid-rotor approximation in the MMX molecular-mechanics calculations[9] program reveal that the bicyclic crown ether has a boat conformation as shown in Fig. 2. When Na^+ or K^+ complexes are formed a conformational change is produced; firstly, the ether moiety containing the cation has a diequatorial oxygen arrangement. Secondly the cyclohexane is in a chair conformation; and consequently the other crown ether has a diaxial conformation that makes it unable to complex any cation.

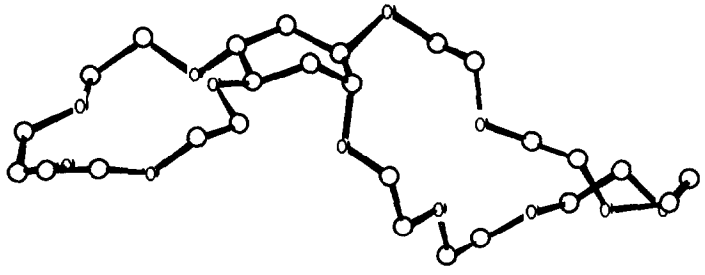


Fig. 2. Simulated structures of **1** computed using the MMX program and drawn by Chem 3D

The boat conformation in **1** must be changed into a chair to complex the alkali cations, and this conformational change involves an energetic cost; this fact could explain the smaller association constants of the bis-cyclic compounds.

Transport of Na^+ and K^+ across a $CHCl_3$ liquid membrane with compounds **1** and **2** was carried out under the conditions described previously[3]. As reflected in Table 2, **14** preferably transports Na^+, and **15** K^+, while **16** Na^+/K^+ transport is around 1, which means that both cations are transported in similar proportions. Experiments using just one cation (K^+ or Na^+) with bicyclic systems **1** and **2** were carried out and in both cases transport was negligible. In K^+/Na^+ systems compound **2** was able to transport Na^+ and K^+ in equivalent amounts and with similar efficiency as

monocyclic ethers, but the behaviour of **1** in K^+/Na^+ transport was quite different: this carrier transported twice as much Na^+ and K^+ than monocyclic compounds or compound **2.**

Table 2. Transport of K^+ and Na^+ (10^{-6}mol cation/mmol carrier)

Carrier	K	Na
CH-5 (**14**)	0.404	0.681
CH-6 (**15**)	0.705	0.413
CH-7 (**16**)	0.574	0.599
5-CH-7 (**1**)	2.160	2.070
6-CH-7 (**2**)	0.894	0.781

As the results in Table 2 show, the hole size is an important factor in cation transport because the compound which has more similar crown ether sizes shows no cooperative effect, and its transports as much as both monocyclic systems together. Compound **1** is an odd carrier and its behavior can be explained only if the negative allosteric cooperativity has an important effect on transport. The behavior of compound **2** could be related to smaller affinity for cation Na^+; this low affinity could prevent the conformational change to the chair necessary to form the complex.

Experimental Section

Preparation of 1,2-Epoxy-4-Cyclohexene 3 and 1,2-Epoxy-Cyclohexane 12. 21.5 g of Na_2HPO_4 were dissolved in 50 mL of 30% hydrogen peroxide. A second phase consisting of 5 mL (49 mmol) of cyclohexane and 5.1 mL (54 mmol) of ethyl chloroformate in 30 mL of dichloromethane was added. The reaction was stirred at room temperature for one day. The phases were separated and the aqueous layer was extracted with dichloromethane. The combined organic fractions were washed with a solution of $NaHSO_3$ and dried with magnesium sulfate. Distillation at reduced pressure afforded 3.3g (68%) of **12** as a colorless liquid. ^1H NMR (200 MHz, $CDCl_3$) 1.30 (m_b, 2H); 1.82 (m_b, 2H); 3.09 (m, 1H). ^{13}C NMR ($CDCl_3$) 19.32, 24.32, 52.07

3 was obtained from 1,4-cyclohexadiene in 80 % yield as a colourless liquid.^1H NMR (200 MHz, $CDCl_3$) 2.45 (m, 2H); 3.19 (s_b, 1H); 5.40 (s_b, 1H); ^{13}C NMR ($CDCl_3$) 24.66, 50.58, 121.19.

Trans-2-Hydroxy-4-Cyclohexenyl and trans-2-Hydroxy Cyclohexyl Ethylene Glycol Ethers 4 and 13. General Procedure. 0.1 mol of the appropriate epoxicompound, 0.2 mol of diethylene glycol and 0.1 mL of sulfuric acid (95 %) in 60 mL of chloroform were heated under reflux for 8 h. The cold reaction was neutralized with 5 % sodium carbonate solution. The organic layer was filtered through silica gel and chromatographed on silicagel with acetone:toluene (1:1). Compound **13** was obtained as a pale yellow oil (30%).^1H NMR (200 MHz, $CDCl_3$) 1.14 (m, 4H); 1.61 (m, 2H); 1.94 (m, 2H); 3.02 (m, 1H); 3.37 (m, 1H); 3.58 (m, 10H). ^{13}C NMR ($CDCl_3$) 24.14, 24.42, 29.88, 32.50, 61.59, 68.62, 70.99, 72.99, 73.89, 84.89.

Compound 4 was obtained in a 28% yield as a pale yellow oil.^{1}H NMR (200 MHz, $CDCl_3$) 2.04 (m, 1H); 2.45 (m, 1H); 3.66 (m, 6H); 5.51 (d, 1H). ^{13}C NMR ($CDCl_3$) 31.08, 33.22, 61.85, 69.12, 70.76, 71.00, 73.04, 81.34, 124.46, 125.27.

Preparation of trans-Cyclohexyl and trans-Cyclohexenyl Crown Ethers. General Procedure. To a solution of the appropriate trans-hydroxyether (5 mmol) in 150 mL of dry THF and under an inert atmosphere, we added potassium tert-butoxide (2.2 equiv). The reaction was heated under reflux for 2 h and then 1 equiv of triethylene glycol ditosylate was added dropwise. The reaction was heated under reflux for 3 days; after this period the solvent was evaporated in vacuo, the residue was suspended in water, and 10% HCl was added until acid pH. The product was extracted into dichloromethane and concentrated in vacuo, and the residue was chromatographed on alumina with ether as eluent to give **3** in 40 % yield as a pale yellow oil.^{1}H NMR (200 MHz, $CDCl_3$) 2.0-2.2 (m, 1H); 2.40-2.55 (d_b, 1H); 3.5-3.9 (m, 11H); 5.52 (d_b, 1H).^{13}C NMR ($CDCl_3$) 30.814, 69.550, 70.502, 70.581, 70,862, 70.963, 78.337, 124.354. Anal. Calcd for $C_{16}H_{28}O_6$: C, 62.74%; H, 8.86%. Found C, 62.61%; H, 8.90%. With tetraethylene glycol ditosylate **6** was isolated in 32 % yield as a yellow oil.^{1}H NMR (200 MHz, $CDCl_3$) 2.4-2.7 (m, 2H); 3.5-4.0 (m, 13H); 5.51 (d_b, 1H). ^{13}C NMR ($CDCl_3$) 69.584, 70.619, 70.687, 70.955, 71.066, 78.394, 124.295. Anal. Calcd for $C_{18}H_{32}O_7$: C, 60.00%; H, 8.88%. Found C, 59.68%; H, 8.79%. **11** was obtained from **9** and diethylene glycol ditosylate in 23 % yield ^{1}H NMR (200 MHz, $CDCl_3$) 2.05 (m, 1H); 3.1-3.5 (m,20H). ^{13}C NMR ($CDCl_3$) 70.863 (broad) Anal. Calcd. for $C_{24}H_{44}O_{11}$: C, 56.69%; H, 8.66%. Found C, 56.67%; H, 8.63% . **1** was prepared from **8** and diethylene glycol ditosylate in 21 % yield as an oil.^{1}H NMR (200 MHz, $CDCl_3$) 0.9-1.15 (m, 1H); 1.7 (m, 1H); 2.7-3.6 (m, 22H). ^{13}C NMR ($CDCl_3$) 70.893 (broad), 30.26. Anal. Calcd for $C_{26}H_{48}O_{12}$: C, 56.52%; H, 8.69%. Found C, 56.26 %; H, 8.91%. MS 306, 133, 95, 89, 81, 73, 45. **2** was obtained from **8** and tetraethylene glycol ditosylate in 42 % yield as an oil^{1}H NMR (200 MHz, $CDCl_3$) 2.12 (m,2H); 3.63 (m, 24H). ^{13}C NMR ($CDCl_3$) 70.839, 29.37, 27.76. Anal. Calcd for $C_{28}H_{52}O_{13}$: C, 56.37%; H, 8.72%. Found C, 56.60 % H,9.09 %. SM 378, 316, 133, 89, 87, 73, 45. **14** was prepared in 23 % yield as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) 1.16 (m,2H); 1.64 (m, 1H); 1.97 (m, 1H); 3.17 (m, 1H); 3.84 (m, 8H). ^{13}C NMR ($CDCl_3$) 23.696, 30.231, 69.124, 70.398, 70.774, 70.942, 82,136. Anal. Calcd for $C_{14}H_{26}O_5$: C, 61.31%; H, 9.49%. Found C, 61.42 %; H, 9.25 %. MS 274, 187, 133, 99, 89, 73, 45. **15** was isolated in 35 % yield as a yellow oil. ^{1}H NMR (200 MHz,$CDCl_3$) 1.1-1.3 (m, 2H); 1.6-1.7 (m, 1H); 1.95-2.05 (m, 1H); 3.1-3.25 (m, 1H); 3.55-3.9 (m, 10H). ^{13}C NMR ($CDCl_3$) 23.757, 30.405, 69.343, 70.584, 70.865, 82.281. Anal. Calcd for $C_{16}H_{30}O_6$: C, 60.37%; H, 9.43%. Found C, 59.98 %; H, 9.27 %. MS 318, 231, 187, 133, 117, 99, 89, 73, 45. **16** was obtained in 25 % yield as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$) 0.98 (m,2H), 1.44 (m, 1H); 1.75 (m, 1H); 2.97 (m, 1H); 3.44 (m, 12H). ^{13}C NMR ($CDCl_3$) 68.960, 69.932, 70.060, 70.236, 70.382, 70.433, 81.684. Anal. Calcd for $C_{18}H_{34}O_7$: C, 59.67%; H, 9.39%. Found C, 59.37 %; H, 9.07 %. MS 362, 275, 133, 117, 99, 89, 73, 45.

Epoxidation of 4-Cyclohexenyl Crown Ethers. General Procedure. To a cold 0.4 mmol of 4-cyclohexenyl crown ether in 20 mL of dichloromethane solution, 1 equiv of 3-chloroperbenzoic acid was slowly added. The reaction was stirred for 1 h at room temperature and

then poured into a 10 % sodium carbonate solution. The aqueous phase was washed with dichloromethane and the organic phases were dried and evaporated to yield the product. Purification on silica gel using dichloromethane:methanol (100:5) afforded **7** in 61 % yield as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) 1.80 (m, 2H); 2.45 (m, 2H); 3.07 (t_b, 1H, J=4.3Hz); 3.14 (m, 1H); 3.34 (m, 2H); 3.32 (m, 20H). ^{13}C NMR ($CDCl_3$) 30.212, 50.700, 53.218, 69.854, 69.991, 70.046, 70.483, 70.665, 70.812, 71.156, 71.229, 76.556, 78.041. Anal. Calcd for $C_{16}H_{28}O_7$: C, 57.83%; H, 8.43%. Found C, 57.98 % H, 8.34 %. Compound **8** was isolated in 62 % yield as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) 1.92 (m, 2H); 2.45 (m, 2H); 3.08 (t_b, 1H, J=4.4Hz); 3.19 (m, 1H); 3.35 (m, 2H); 3.65 (m, 24H). ^{13}C NMR ($CDCl_3$) 30.510, 50.783, 53.367, 70.285, 70.527, 70.645, 70.839, 70.972, 71.041, 76.872, 78.320. Anal. Calcd for $C_{18}H_{32}O_8$: C, 57.44% ; H, 8.51%. Found C, 57.41% ; H, 8.52%.

Preparation of Hydroxyethylene Glycol Ether of Cyclohexyl Crown Ethers. General Procedure. The appropriate 4,5-epoxicyclohexyl crown ether was dissolved in 30 mL of dry chloroform and 2 equiv of ethylene glycol with 50 mg of concentrated sulfuric acid were added. The mixture was refluxed under inert atmosphere for one day. After quenching with sodium carbonate the product was extracted into dichloromethane and concentrated in vacuo, and the residue was chromatographed on alumina with ether. **9** was afforded in 40 % yield as a oil.^{1}H NMR (200 MHz, $CDCl_3$) 1.3-1.5 (m, 2H); 2.9 (s_b, 1H) 3.5-3.7 (m, 16H).^{13}C NMR ($CDCl_3$) 32.079, 61,649, 70.181, 72.260, 76.340, 76.977, 77.614. **10** was obtained in 30 % yield as a oil. ^{1}H NMR (200 MHz, $CDCl_3$) 1.4-1.7 (m, 2H); 3.3 (s_b, 1H); 3.6-3.8 (m, 18H). ^{13}C NMR ($CDCl_3$) 32.102, 61.894, 70.201, 72.279, 72.879, 77.092.

Cation Transport Studies

Membrane transport experiments were carried out with an $H_2O/CHCl_3/H_2O$ bulk liquid membrane system. The metal chlorides were obtained from commercial sources in the highest grade available and were used without further purification. The metal solutions were prepared with distilled deionized water.

Source phases were prepared from appropriate amounts of MCl. After 3 days the receiving phase was sampled and analyzed for cation concentration using a Perkin Elmer 2380 atomic absorption spectrometer (acetylene-air flame). Each experiment was repeated at least three times, and the results reported in Table I are the overage of the three determinations. The standard deviation from the mean value among the data in each experiment is 15 %.

Determination of given Association Constants by Ultraviolet Method._ All ultraviolet measurements were made with a Spectrometer Shimadzu UV-240 at 380 nm. at 24-26°C. Typically, 5 to 7 complexation experiments were run simultaneously with a given host. Picrate salts in distilled water were prepared with concentration 0.010M. Solutions of the hosts 0.075M in $CHCl_3$ were also prepared.

1.0 mL of the picrate solution was introduced in a tube . To one tube we added 1.0 mL of water to be used as a blank. To each of the tubes, including the one containing water, we added 1.0

mL of the host solution. The contents of each tube were then stirred vigorously for 3 min. with a magnetic stirrer, and separated into clear layers by centrifugation.

An aliquot of 100 μL of the $CHCl_3$ layer was transferred by microsyringe into a 10-mL volumetric flask and diluted with CH_3CN. For each size of aliquot a blank was also made by measuring the desired volume from the $CHCl_3$ layer of the H_2O blank and diluting with CH_3CN in a 10-mL volumetric flask. The UV absorption of each solution was measured against the appropriate blank solution at 380 nm. The absorbance of the sample cell at 380 nm. relative to the absorbance of the blank cell when both were filled with CH_3CN was measured prior to each series of extractions. Calculations were based on Beer's law relationship and on Cram's equation. Extinction coefficients for each salt in CH_3CN were determined in the range of 10^{-4}-10^{-6}M of standard solutions prepared directly from the pure salts. The average was used in the calculations.

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