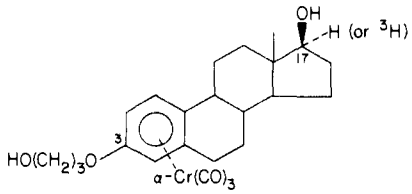

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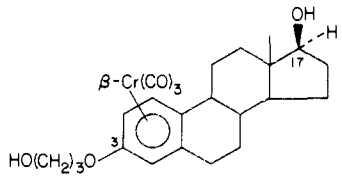

2

In order to prove the specific association of the chromium-labeled steroid for the estradiol receptor site, we prepared compound **1** tritium labeled at the 17α-position[12] and performed in vitro incubation experiments with lamb uterine cytosol (Table I). In this way, we were able to demonstrate that the radioactive hormone is bound specifically and reversibly to the uterine estrogen receptor. Furthermore, the amount of nonspecific binding is only slightly increased when compared to that of (^{3}H)estradiol itself. We also checked that compound **1**, as well as estradiol, binds poorly to, and is not displaced by DES from, high-capacity, low-affinity proteins found in nontarget tissues (e.g., rat lung). That the organometallic label does not decompose during the binding experiments is indicated by the following Fourier transform infrared (FT-IR) measurements.[13]

Figure 1a shows that FT-IR spectrum of lamb uterine cytosol used as a source of estradiol receptor following incubation with 17β-estradiol and precipitated from the cytosol by protamine sulfate technique.[11] The FT-IR spectrum of compound **1** is given in Figure 1b; the two ν(CO) peaks characteristic of the C_{3v} symmetry Cr(CO)$_3$ fragment are the strongest absorption present. Next, in Figure 1c, we show the FT-IR spectrum of the precipitated proteins following incubation with the organometallic tritiated compound **1** at approximately the same concentration ($\sim 10^{-8}$ M) as currently used in the radiochemical assays using estradiol itself.[6] The protein absorption are off-scale owing to the thickness of the minipellet but two very weak features can be discerned above the background at $\sim$1900 cm^{-1}. The computer expansion of this region shown in Figure 1d reveals the two ν(CO) peaks of the organometallic marker. Similar results were obtained with all the chromium tricarbonyl labeled estradiol molecules synthesized in this work. While the best signal-to-noise conditions for the spectra necessitated recording 10 000–30 000 scans at 4-cm^{-1} resolution (3–10 h), the two metal carbonyl peaks can just be detected above the background at 8-cm^{-1} resolution in $\sim$2 min. There is an excellent correlation (R = 0.98) between the area of the higher energy ν(CO) peak in the experiments with compound **1** and the weight of the minipellets, indicating that in principle it should be possible to extend this new method of protein receptor detection into the quantitative realm in the future.

Acknowledgment. This research was generously supported by operating grants from C.N.R.S. and P.I.R.M.E.D. (France), N.S.E.R.C. (Canada), and F.C.A.C. (Quebec) and travel grants under the auspices of a France–Quebec Exchange.

Registry No. 1, 93061-16-6; **2**, 93061-17-7; [17α-*H*]-**1**, 96648-81-6; **4**, 96648-82-7; **5**, 96687-90-0; 17β-estradiol, 50-28-2.

(12) As direct synthesis of ([6,7-^{3}H]-17β-estradiol)tricarbonylchromium complexes failed owing to radiolysis, [17α-^{3}H]-**1** was prepared by reduction with [^{3}H]NaBH$_4$ of the [3-*O*-(3-hydroxypropyl)estrone]tricarbonyl complex **4** obtained as follows: Estrone was heated with NaOH and Br(CH$_2$)$_3$OH in acetone.[9] After complexation by heating with Cr(CO)$_6$ 4$^1/_2$ h at reflux in *n*-Bu$_2$O under argon and separation of the diastereomers (TLC using ether/pentane, 10/1) compounds **4** (estrone analogue of **1**, mp 97 °C, 12%) and **5** (estrone analogue of **2**, mp 94 °C, 12%) were obtained. [17α-^{3}H]-**1** was obtained by heating 25 μM of **4** (20 h at 50 °C in 1.2 mL of 0.1 N NaOH/isopropyl alcohol, 1/5) in the presence of 6.25 μM of [^{3}H]NaBH$_4$ (Amersham, England, S.A. 16 Ci/mol). After hydrolysis, purification (TLC using ether), and crystallization (ether/pentane), the yellow solid obtained (0.64 mg) was identified as authentic compound **1** with a specific activity of 4.58 Ci/mol.

(13) FT-IR spectra were recorded on a Nicolet 6000 spectrometer equipped with a mercury–cadmium–telluride, liquid nitrogen cooled detector. Samples (1.5–2.0 mg) of the dried, white powders obtained from the protein precipitations of the cytosol were pressed into 3-mm minipellets.

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Bromate Oscillators: Elucidation of the Source of Bromide Ion and Modification of the Chemical Mechanism

Margit Varga, László Györgyi, and Endre Körös*

Institute of Inorganic and Analytical Chemistry
L. Eötvös University, H-1443 Budapest, Hungary

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Although the FKN mechanism[1] is widely accepted, it is still debated which chemical reactions produce the bromide ion, the control intermediate of BZ oscillators. According to the FKN mechanism, bromide ion is generated in a reaction between the oxidized form of the catalyst, $M^{(n+1)+}$, and bromomalonic acid (BrMA) accumulated during the preoscillatory period. This multistep reaction appears in a concise form in step 5 of the Oregonator model:[2] $Z \rightarrow fY$ ($2Ce^{4+} \rightarrow fBr^-$).

This assumption has been supported by the observation that in a BZ system chemical oscillation starts only after the concentration of BrMA reaches a crucial value.[3] On the other hand, a number of experimental results cannot be reconciled with the above supposition. Some of these are the following: (a) in a BZ system with a high [MA]/[BrO_3^-] ratio (>30) oscillation starts without a preoscillatory period;[4] (b) there are many BZ systems where bromide ion cannot be formed by a reaction between $M^{(n+1)+}$ and an organic bromo compound because the $M^{(n+1)+}$ + >CHBr $\rightarrow M^{n+}$ + Br^- + CO_2 ... reaction is either slow or does not proceed at all, e.g., when the organic compound is malic acid or an aliphatic or cyclic diketone; (c) in the presence of bromo-complex-forming metal ions, e.g., Tl^{3+}, which complex the bromide ions generated in the BZ reaction, the rates of BrMA and bromide formation are practically equal;[5] (d) the measured rate of carbon dioxide evolution is 2 orders of magnitude higher than that calculated from the BrMA–Ce^{4+} reaction.[6]

Owing to problems concerned with the stoichiometric factor, f, of the Oregonator model, a few authors have looked for additional sources of bromide ion, i.e., for other reactions that may produce bromide ion. They assumed that bromide ion forms *also* in the reduction of oxybromine compounds.[7] A conclusion to this question, however, has not been given so far.

In order to understand this crucial point of the mechanism of BZ oscillators, we have performed reactions in BZ systems that contain both ^{82}Br-labeled BrMA and silver ions. In the starting reaction mixture the concentration of BrMA was above the crucial value[3] and equal with that of Ag^+. The initial composition of the system was the following: 0.08 M $KBrO_3$, 0.20 M malonic acid,

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0002-7863/85/1507-4780$01.50/0

Table I

step		k_+, M^{-1} s^{-1}	k_-, M^{-1} s^{-1}	ref
1	$Br^- + BrO_3^- \rightleftharpoons HOBr + HBrO_2$	$2.1[H^+]^2$	10^5	8
2	$Br^- + HBrO_2 \rightleftharpoons 2HOBr$	$2 \times 10^9[H^+]$	5×10^{-5}	8
3	$HBrO_2 + BrO_3^- \rightleftharpoons 2BrO_2$	$1 \times 10^4[H^+]$	2×10^7	8
4	$BrO_2 + Ce^{3+} \rightleftharpoons HBrO_2 + Ce^{4+}$	$6.5 \times 10^5[H^+]$	2.4×10^7	8
5	$BrO_3^- + Ce^{3+} \rightleftharpoons BrO_2 + Ce^{4+}$	$1.3 \times 10^{-4}[H^+]$	9.1	8
6	$2HBrO_2 \rightleftharpoons BrO_3^- + HOBr$	4×10^8	$2.1 \times 10^{-10}[H^+]$	9
7	$HOBr + Br^- \rightarrow Br^-$	$6.55 \times 10^7[H^+][MA]$		10
8	$Ce^{4+} + RH \rightarrow Ce^{3+} + R\cdot$	1.0		8
9	$2R\cdot \rightarrow RH + ROH$	9.9×10^8		8
10	$ROH + BrO_3^- \rightarrow Br^-$	5.0		
11	$Ce^{4+} + ROH \rightarrow Ce^{3+}$	2.13		8

Table II. Comparison of the Measured and Simulated Period Times and SBPPs[a]

[MA], M	$[Ce^{4+}]$, M	time of one oscillation, s		SBPP, s	
		measured	simulated	measured	simulated
0.10	0.001	66	67.2	16	11
0.10	0.0005	66	65.5	18	14
0.20	0.001	48	46.2	7.5	7
0.50	0.001	31	37.1	3	4

[a] $[BrO_3^-] = 0.06$ M; $[H_2SO_4] = 1.0$ M.

1.0 M H_2SO_4, 0.01 M ^{82}BrMA, 0.01 $AgNO_3$, and 0.002 M Ce^{4+}. Bromide ion produced during the BZ reaction was precipitated as AgBr. The reaction was allowed to proceed until all of the silver ion was converted to AgBr (about 10–15 min). The latter was separated by filtration, dissolved in a KCN solution, and diluted to a given volume. The activity of both this solution and that of the filtrate was measured on a well-type γ-scintillator.

If bromide is produced from BrMA then the AgBr should be active, if bromate is the source, either directly or undirectly, of bromide, then the AgBr should be inactive. Three parallels each of two runs gave the following results: 6.5% and 7.3%, respectively, of the total activity appeared in AgBr and 93.5% and 92.7%, respectively, was found in the filtrate.

We did not find bromine atom exchange between BrMA and bromate ion.

Our results clearly show that in reacting BZ systems the source of bromide ion is predominantly bromate ion. This recognition prompts us to modify the chemical mechanism. As shown below, we have bromide ion being generated in a reaction between bromate ion and an oxidation product of malonic acid (ROH). The stoichiometry thus obtained agrees with the most favorable stoichiometry ($f = 1$) of the Oregonator.

The reacting BZ system is simulated by the mechanistic steps and rate constants in Table I. The value of k_{10} proposed by us seems to be reasonable for a reaction between bromate ion and a loosened alcoholic hydroxyl group. The rate-determining step is the direct attack of bromate ion on HOCH<.[11] (We are aware of that there are other possible reaction routes for the regeneration of bromide ion. We shall analyze these in the forthcoming publication. In the computational studies we intend to use not only the "high" but also the "low" sets of rate constants.[12])

Data presented in Table II show that not only the experimental period time but also the slow bromide production period (SBPP) can be simulated. Figure 1 shows the simulated bromide concentration and redox potential vs. time curves.

Since in the proposed mechanistic model bromide ion is generated not in a reaction between $M^{(n+1)+}$ (e.g., Ce^{4+}) and an organic bromo compound, it seems to be applicable to BZ systems containing organic compounds other than malonic acid as well as for uncatalyzed bromate oscillators.

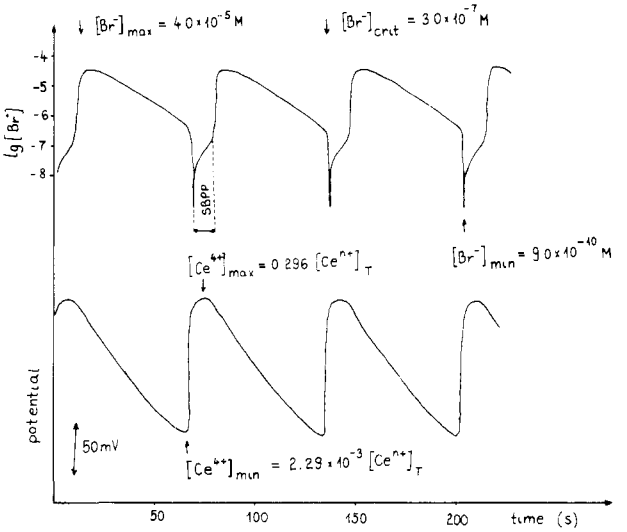

Figure 1. Simulation of the bromide concentration and redox potential vs. time curves by the modified chemical mechanism. The composition of the system: $[BrO_3^-] = 0.06$ M, $[H^+] = 1.0$ M, [MA] = 0.10 M, and $[Ce^{4+}] = 0.001$ M.

Registry No. MA, 141-82-2; BrO_3^-, 15541-45-4; Br^-, 24959-67-9; Ce, 7440-45-1.

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Synthesis of the First α-Diazo Phosphines. Phosphorus–Carbon Multiple-Bond Character of Phosphinocarbenes

Antoine Baceiredo, Guy Bertrand,* and Ghislaine Sicard

Laboratoire des Organométalliques, U.A. 477, Université Paul Sabatier, 31062 Toulouse Cedex, France

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It is well established that aminonitrene,[1] sulfenylnitrene,[2] sulfinylnitrene,[3] and phosphinonitrene[4] can be regarded as diazene,

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