

Study the Interaction between Prolactin Hormone and some Organotellurium Compounds.

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Abstract:

In the present study , three organotellurium compounds have been prepared by a simplified methods. These compounds include; diphenyl ditelluride (compound1), Bis(p-methoxy phenyl) ditelluride (compound3), and p-methoxy phenyl tellurium trichloride (compound2). These compounds have been prepared from reaction TeCl_4 as starting material with some ethers in different solvents. All these organotellurium compounds were characterized the well-known methods including: FTIR, UV-Visible spectrophotometry, CHN analysis, melting point and ^1H NMR spectroscopy.

The study of prolactine hormone adsorption on the surface of these compounds showed that the equilibrium time of adsorption was between 30-60 minutes. Prolactin concentrations were followed by enzyme-linked immunosorbent assay (ELISA) technique. Tellurium compounds extract good amounts of prolactin from solution on their surfaces and the differences between the absorbed quantities are small. All absorption process curve were of S-shaped according to Gills classification. The adsorption isotherm studies showed that most Te compounds obeyed Frundlish equation isotherm indicating the heterogeneity of the energy of the active sites on the organotellurium compounds surface. Desorption studies show a low desorption percentage, which indicates a strong association between Prolactin and organotellurium compounds.

These properties can be used to immobilize proteins on the surfaces of these compounds as a useful tool in the biochemical researches or in the extraction processes of minute amounts of important proteins from the biological fluids.

Introduction

Organotellurium compounds chemistry describes the synthesis and properties of chemical compounds containing a carbon-telluriumchemical bond⁽¹⁾. These compounds have many applications and dimethyl telluride is one of the organotellurium compound that has been quantified in environmental as a product of microbial metabolism⁽²⁾. Organic tellurides have been used as initiators for living radical polymerization and electron-rich mono- and di-tellurides possess antioxidant activity. It also used rubber can be vulcanized with tellurium instead of sulfur or selenium. The rubber produced in this way shows improved heat resistance⁽³⁾.

Organotellurium compounds play an important role in organic synthesis, and they have received considerable attention because of their potential availability and useful biological activity⁽⁴⁾. Diphenyl ditelluride [(PhTe)₂], an organochalcogen compound, is a teratogenic agent causing various morphological abnormalities in rat fetuses in development⁽⁵⁾.

The present study has three aims. First, is the preparation and identification of some organotellurium compounds in simple modified methods. The second aim is to study the ability of the prepared compounds to interact with the prolactin hormone as a mean to extract or immobilize the prolactin hormone on the surface of these compounds. The third aim is to study the surface properties of these compounds from the results of the adsorption-desorption results of the interaction between the prolactin and organotellurium compounds.

Materials and Methods

1- UV-Visible Spectrophotometer

The scanning spectra were measured using UV-Visible spectrophotometer (Shimadzu-UV-1700) to obtain the maximum wave length of the absorption of the compounds while the absorbances of the solutions were measured using Spectrophotometer type (Shimadzu-UV-1700).

2-Melting Point Apparatus

Melting Point of the prepared compounds were recorded using Electrothermal melting point apparatus, UK.

3-Infrared Spectrophotometer

FTIR spectra were recorded by using Fourier transformed infrared spectrophotometer (SHIMADZU FTIR-8400S) using KBr disc as a solid support.

4-Analysis of the Elements (CHN)

The elemental analyses (CHN) were recorded using (EURO Elemental Analyzer) at the Central Laboratory-University of Kufa.

5-¹H-Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) spectra were recorded using Fourier Transform Varian Spectrometer operating at 300MHz with tetramethylsilane as internal standard. Measurements were made at the Department of Chemistry, Ahl Al-Bayt University, Jordan.

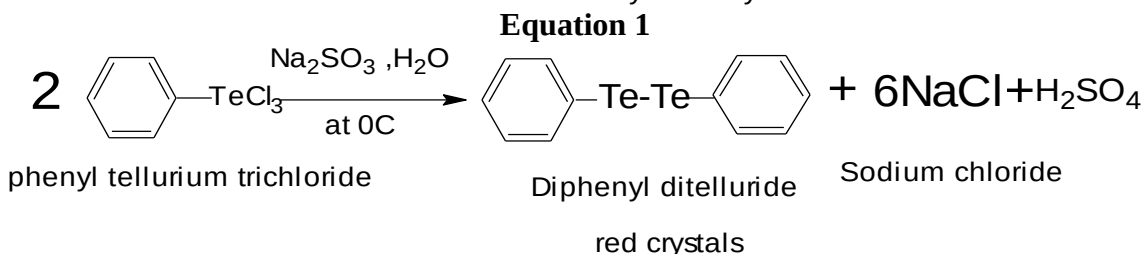
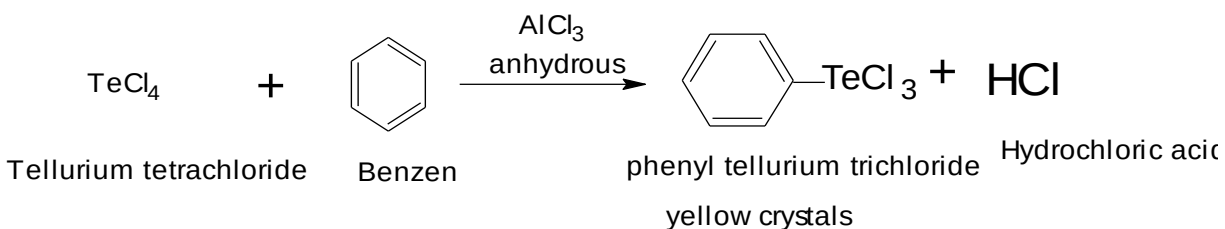
6-ELISA Reader

ELISA reader (M+, USA) was used to measure the intensity of coloured solutions in microwells at fixed wavelength (450nm).

A-Preparation Organotellurium Compounds

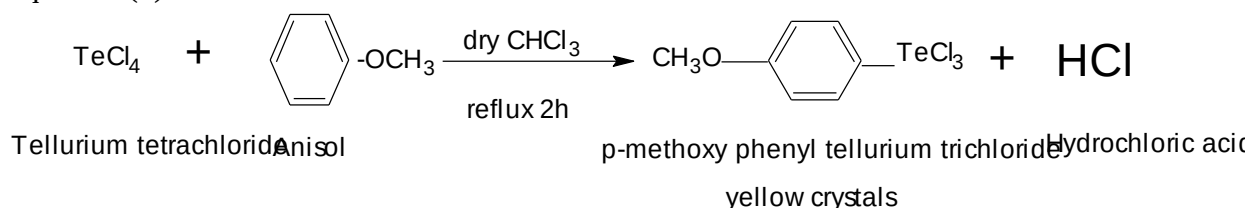
1-Diphenyl ditelluride

Diphenyl ditelluride (compound 1) was prepared from tellurium tetrachloride (B.D.H) and dry benzene and reflux with heating for 15-20 minute according to preparation phenyl tellurium trichloride compound, the second stage reduction phenyl tellurium trichloride to preparation diphenyl ditelluride to the following chemical Equations (1 and 2) ⁽⁶⁾:



Equation 2

2-p-methoxy phenyl tellurium trichloride
p-methoxyphenyltellurium trichloride was prepared according to the following chemical Equation (3)⁽⁷⁾:

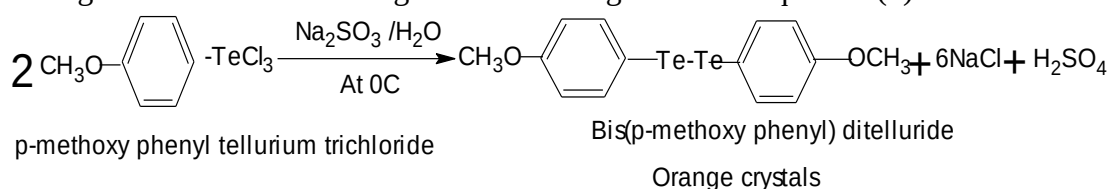


Equation 3

P-methoxy phenyl tellurium trichloride (compound 2) was also prepared from tellurium tetrachloride and anisole in dry chloroform with heating and reflux for 2 hours⁽⁷⁾.

3-Bis(p-methoxy phenyl)ditelluride

Bis(p-methoxyphenyl) ditelluride (compound 3) was prepared from reduction of two molecules p-methoxy phenyl tellurium trichloride by Sodium Sulfate in water with continuous stirring for 45 minute according to the following chemical Equation (4)⁽⁷⁾:



Equation

4

B-Estimation of Prolactin Concentration by ELIS

C-Adsorption study

To study the adsorption isotherms of the adsorption of prolactine on organotellorium compounds, 10µl of prolactin solution at concentrations 0, 5, 10, 25, 50 and 100ng/ml was added to 50mg of each organotellurium compounds in an Eppindroff® tubes. The tubes were incubated with shaking for one hour which is the more than the equilibrium time obtained from a separate experiments. The prolactin concentration in the aspirated supernatant by enzyme Linked immunosorbent assay ELISA technique^(8,9) using Kits supplied by monobind Co. USA . Before measurement, the tubes were centrifuged at 3000rpm for 5 minutes to precipitate the organotellurium compounds.

The amount of prolactin adsorbed was calculated from by the following equation:

$$Q_e = \frac{x}{m} = \frac{V(C_0 - C_e)}{m} \dots\dots\dots (II-1)$$

X: The quantity adsorbed (ng), V: Volume of solution (ml), Co: Initial concentration (ng/ml), Ce: Equilibrium concentration (ng/ml), m: Weight of adsorbent (Organotellurium compound)

Qe values were plotted versus the equilibrium concentrations (Ce), the resulting diagrams are the adsorption isotherms that required for understanding and interpreting the systems under investigation.

D- Desorption process:

To study the strength of the prolactin-organometallic interaction, the desorption experiments were carried out. In each case, prolactin was adsorbed from solution onto the organometallic compounds using a protocol similar to that for the adsorption using one initial prolactin concentration (100µl) for each organometallic compound. After incubation and centrifugation as above described, the supernatant was removed and replaced by 100µl of normal saline solution (0.9%NaCl) to prevent prolactin denaturation. The samples were further incubated at the room temperature to allow desorption to occur. Before measurement, the tubes were centrifuged at 3000rpm for 5 minutes to precipitate the organotellurium compounds and the prolactin level was estimated in the aspirated supernatant by ELISA technique. The desorption percentages were calculated from the ratio between the prolactin quantity released into the solution on the prolactin quantity desorbed initially on the organometallic compound i.e., weight of PRL in 100µl/weight of PRL in 50mg:

$$\% \text{Desorbed} = \frac{C_{e/10}}{C_{e/20}} * 100\% \dots\dots\dots (1)$$

Results & Discussion

1-Identification of Organotellurium Compounds

Organotellurium compounds was identified by ¹H-NMR , FTIR , UV-vis spectroscopy ,C.H.N. analysis and melting point.

Table (1): Analytical data, physical properties and yields for three compounds

Compounds	Color	Solubility in water	M.P°C	Yield%	Analysis% Measured (Calculated)	
					C%	H%
Diphenyl ditelluride	Red Solid	insoluble	65	50%	35.52 (35.29)	2.12 (2.45)
p-methoxy phenyl tellurium trichloride	Yellow solid	insoluble	197	90%	24.16 (24.77)	1.73 (2.06)
Bis(p-methoxy phenyl)ditelluride	Orange solid	insoluble	67	75%	41.97 (41.86)	2.33 (2.71)

The UV-visible spectrum of compounds 1 and 3 showed the appearance of a band at 400nm⁽¹⁰⁾ this data also means the transition of $n \rightarrow \pi^*$ within the aromatic ditelluride. Furthermore, the UV-visible spectrum of compound 2 shows the disappearance of band at 400nm due to the diorganoditelluride⁽¹⁰⁾ and the appearance of new band at 310nm indicating the formation of organotellurium trichloride⁽¹¹⁾.

The FTIR spectrum for (compounds 1) diphenyl ditelluride, shows the appearance of stretching vibration (3030cm^{-1}) which is due to the (C-H) of aromatic ring. The absorption of two bands at (1433cm^{-1}) and (1470cm^{-1}) is due to the stretching vibration of (C=C) of aromatic ring, while the absorption of two bands at (695cm^{-1}) and (770cm^{-1}) is due to the bending vibration of (C-H) aromatic out of plane⁽¹²⁾, also show the absorption band at (470cm^{-1}) is due to the stretching vibration of (Te-C)⁽¹³⁾.

The FTIR spectrum of (compound 2) p-methoxy phenyl tellurium trichloride, shows the appearance of an absorption band at (3070cm^{-1}) which is due to the stretching vibration (C-H) aromatic ring, appearance of absorption band at (2855cm^{-1}) is due to stretching vibration of the (C-H) aliphatic group. Two strong bands absorption at (1498cm^{-1}) and (1518cm^{-1}) are due to the stretching of the (C=C) aromatic ring. The absorption two strong bands at (1261cm^{-1}) and (1298cm^{-1}) are due to the asymmetric stretching vibration of the (C-O) ether group⁽¹⁴⁾ and also appearance bands at (477cm^{-1}) and (488cm^{-1}) are due to the stretching vibration of (Te-C) and (Te-Cl)⁽¹⁴⁾ respectively.

FTIR spectrum of the (compound 3) Bis(p-methoxy phenyl) ditelluride, shows the appearance of the absorption band at more than (3030cm^{-1}) which is due to stretching vibration of (C-H) aromatic ring and the absorption band at (2900cm^{-1}) is due to stretching vibration of (C-H) aliphatic group. FTIR spectrum also showed the appearance of two absorption bands at (1487cm^{-1}) and (1587cm^{-1}) are due to stretching vibration of (C=C) aromatic ring. The absorption bands at (1176cm^{-1}) and (1260cm^{-1}) are due to the asymmetric stretching vibration of ether group⁽¹⁵⁾. The FTIR spectrum show the is also show the absorption band at (470cm^{-1}) is due to the stretching vibration of (Te-C)⁽¹⁴⁾. ¹H-NMR spectrum, of (compound 1) diphenyl ditelluride, showed the singlet signals at $\delta(6.37\text{ppm})$, $\delta(7.18\text{ppm})$ and $\delta(7.26\text{ppm})$ that could be attributed to the ten protons of aromatic rings in (ph-Te)₂ group^(16,17).

¹H-NMR spectrum of, (compound 2) p-methoxy phenyl tellurium trichloride, shows the doublet signals at $\delta(7.34)$ and $\delta(7.39)$ which refer to the aromatic ring, and the singlet signal seen at $\delta(3.44)$ is due to the methoxy group⁽¹⁸⁾.

¹H-NMR spectrum of (compound 3) Bis(p-methoxy phenyl)ditelluride, showed the two singlet signals at $\delta(3.25\text{ppm})$ and $\delta(3.44\text{ppm})$ which could be attributed the methoxy group and signals at $\delta(6.53\text{ppm})$, $\delta(6.59\text{ppm})$, and $\delta(7.18\text{ppm})$ that could be attributed the aromatic rings⁽¹⁸⁾.

2-Adsorption Studies:

After incubation of prolactin solution with the solid tellurium compounds, a decrease in the absorbencies (i.e., Concentration of prolactin solution) noticed indicating the occurrence of the adsorption process of prolactin from solution on the surface of the Te compounds significantly. The change in the prolactin concentration showed a mild difference among Te compounds. These results indicated a mild dependence of the adsorption process on the organic part of the Te compound as seen in the previous section. To obtain more clear explanation about the adsorption phenomenon of prolactin on Te compounds, the protein adsorption laws and principles are applicable on prolactin as a protein compounds. Proteins are not simply hard spherical colloidal particles. Because of this, several authors have considered changes in orientation, conformation and formation of two-dimensional structures to explain observed adsorption phenomena. The quantities of prolactin (Co=100ng/ml) adsorbed on the surface of Te compounds also this experimental showed a little difference between the quantities of prolactin adsorbed on different Te compounds. Same explanation and discussions are applied for this insignificant difference.

The behavior of the changes of the quantities adsorbed as the concentration changes according to Giles classification⁽¹⁹⁾ indicated that the adsorption isotherms of all three compounds are obey of S1 type. S-Type refers to the fact that; in the initial part of the S curves, however, the opposite condition applies, and the more solute that is already adsorbed, the easier it is for additional amounts to become fixed. This implies a side-by-side association between adsorbed molecules, helping to hold them to the surface. This has been called “ co-operative adsorption ”⁽²⁰⁾. i.e. adsorption becomes easier as concentration rises. In practice, the S curve usually appears when few conditions are fulfilled: the solute molecule has moderate intermolecular attraction, causing it to pack vertically in regular array in the adsorbed layer, and meets strong competition, for substrate sites, from molecules of the solvent or of another adsorbed species.

In order to clarify the surface forces homogeneity and the interaction behavior of the Te compound surface with prolactin, the adsorption isotherms were constructed using Langmuir (Figure 1,2&3) and Freundlich models (Figure 4, 5, &6). Langmuir and Freundlich *isotherms* were used to interpret the *adsorption* data of the investigated systems. When the adsorption data obey the linear form of any equation, this means that the adsorption process obeys the best-fit model i.e., the higher the correlation coefficient value (r), and the adsorption process tends to obey it.

3-Desorption Processes:

The percentage of the quantities of prolactin desorbed from the surface of Te compound were measured from division of the quantity released into the 100µl of solvent (Ce/10) on the adsorbed quantity of prolactin on the 50mg of complex surfaces (Qe/20) i.e.

$$\% \text{Desorbed} = [(Ce/10)/(Qe/20)] * 100\%.$$

Compound 2 showed the highest desorption percentage and in contrast to the Te compound 1 that showed the lowest desorption percentage (Figure 7). The highest the desorption percentage, the lowest energy of adsorption and lowest attraction forces. The adsorption on solid surfaces is often partially, if not totally, irreversible, particularly on hydrophobic surfaces⁽²¹⁾ Hence, when a layer of adsorbed macromolecules is brought in contact with a pure solvent or buffer, no (or only small) desorption is observed over experimental time scales extending over hours or days. This is a direct consequence of the number of interaction points that a protein can establish with the chemical groups of a given surface. Even if the energy associated with each link is low, the overall interaction energy between the protein and the surface can become very important⁽²²⁾, particularly if the mean residence time of the protein increases⁽²³⁾, allowing for the increase in the number of interaction points by means of structural modifications in the adsorbed protein. Thus, the desorption probability should be rigorously described by means of memory functions⁽²⁴⁾. All the links between the adsorbed protein and the surface have to be broken for allowing desorption. The activation energy for such a process may be very high⁽²⁵⁾, rendering the desorption rate constant very low, typically of the order of 10^4 to 10^6 , depending strongly on the protein, the surface, and the buffer used⁽²⁶⁾ Moreover, lateral interactions between the adsorbed proteins may render the desorption process non-Poissonian⁽²⁷⁾ i.e., it cannot be fitted by an exponential or a sum of exponential functions.

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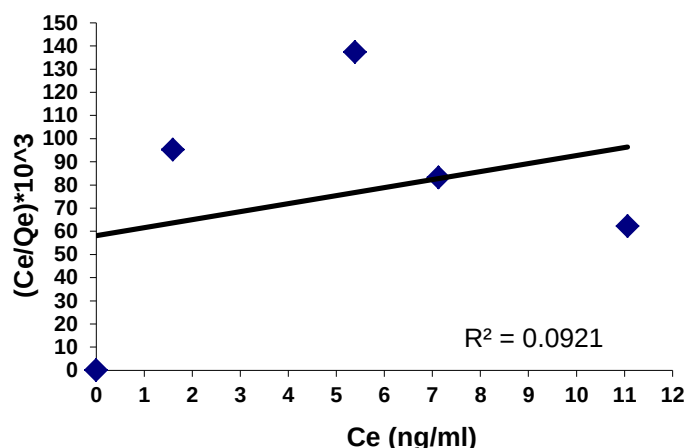


Figure (1): Langmuir Lines of the adsorption of prolactin on the Te compound 1.

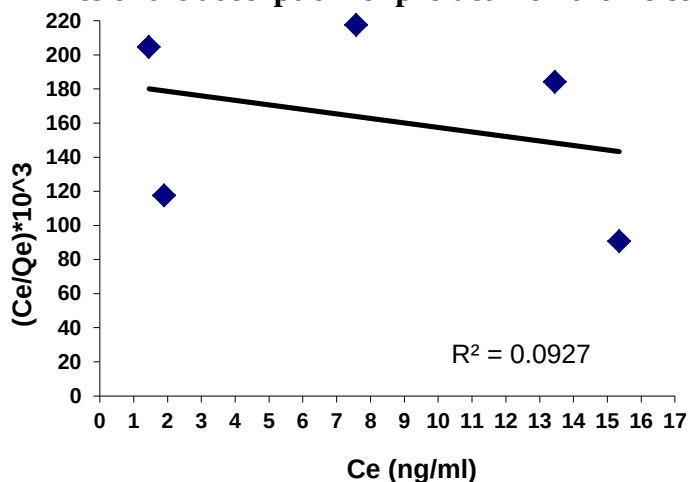


Figure (2): Langmuir Lines of the adsorption of prolactin on the Te compound 2.

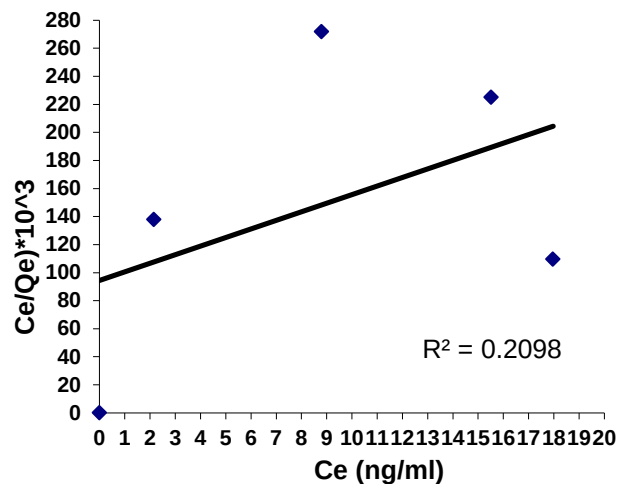


Figure (3): Langmuir Lines of the adsorption of prolactin on the Te compound 3.

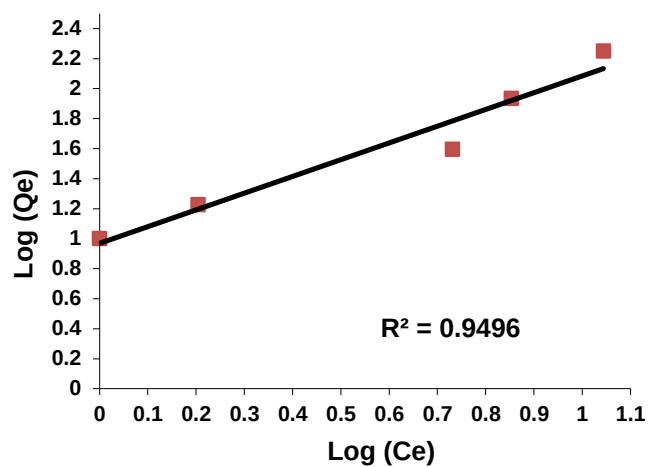


Figure (4): Freundlich Lines of the adsorption of prolactin on the Te compound 1.

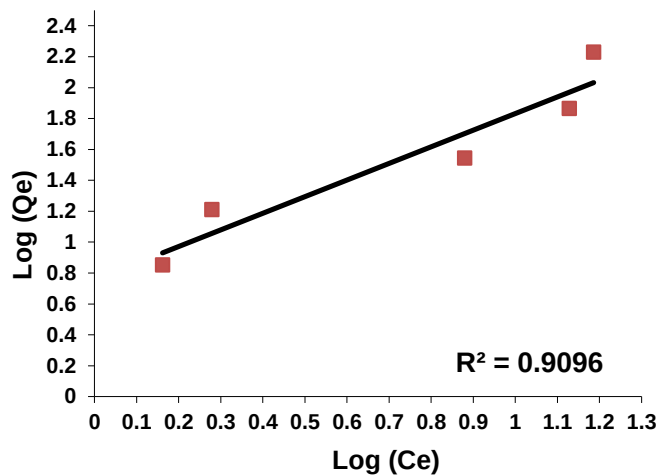


Figure (5): Freundlich Lines of the adsorption of prolactin on the Te compound 2.

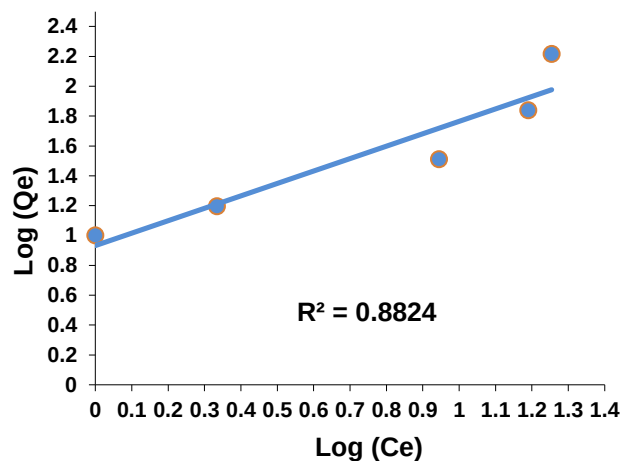


Figure (6): Freundlich Lines of the adsorption of prolactin on the Te compound 3.

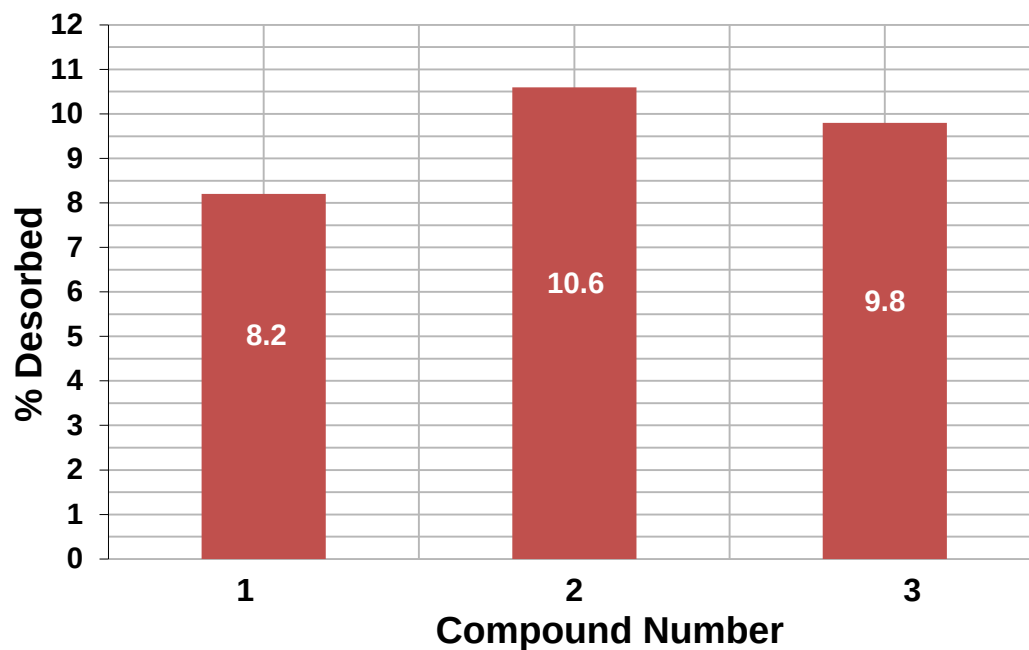


Figure (7): Desorption percentage of the release of prolactine hormone from the the surface of organotellorium compounds.

الخلاصة

تم من خلال هذا العمل تحضير ثلاثة مركبات تلوريوم عضويه باستخدام طرق مبسطة وتشمل: ثنائي فنيل ثنائي التلوريوم (المركب 1) , بس (بارا-ميثوكسي فنيل) ثنائي التلوريوم (المركب 3), وبارا-ميثوكسي فنيل تلوريوم ثلاثي الكلور (المركب 2). حضرت هذه المركبات من تفاعل رابع كلوريد التلوريوم كماده اساس مع بعض الايثرات في مذيبات مختلفة. ان جميع المركبات المحضرة شخصل بطرق معروفة تتضمن: مطيافية الاشعة تحت الحمراء, مطيافية الاشعة فوق البنفسجية, تحليل العناصر الدقيق (كاربون, هيدروجين, نيتروجين), درجات الانصهار و الرنين النووي المغناطيسي.

أشارت دراسة امتزاز هرمون البرولاكتين على سطوح هذه المركبات المحضرة الى ان زمن التوازن للبرولاكتين الممتاز على سطوح هذه المركبات كان بين 30-60 دقيقة. تمكنت مركبات التلوريوم من استخلاص كمية جيدة من هرمون البرولاكتين من المحلول وكان الاختلاف قليل بكمية الهرمون الممتاز على سطوح هذه المركبات كما اظهرت الدراسة ان كل منحنيات عمليات الامتزاز اتخذت شكل حرف S حسب تصنيف كيلز حيث تنطبق معادلة فرنلش على معظم عمليات الامتزاز مما يدل على عدم تجانس القوى المواقع الفعالة على سطوح هذه المركبات, كما اشارت دراسات الابتزاز الى قلة النسبة المئوية للابتزاز مما يدل الى قوة الارتباط العالية بين البرولاكتين وسطوح مركبات التلوريوم.

يمكن الاستفادة من هذه الخصائص في تثبيت البروتينات على سطوح هذه المركبات لاجراض البحوث الحياتية او في استخلاص البروتينات القليلة التركيز من المحاليل البايولوجية.