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A Study with Rivanol

Rivanol ile bir Çalışma

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As it was established in a previous paper (1), there is an incompatibility between rivanol and polyethylene type vehicles. The color of these type mixtures darkens by time.

In this paper the incompatibility between rivanol and one type of the above mentioned vehicles namely polyethylene glycol 4000 was studied in three different angles.

1 — The conditions that might cause the change of the color and at the same time the composition of the mixture.

2 — The type of the new compounds that formed in the mixture.

3 — The difference between the antibacterial activites of the new formed compounds and the original mixture.

EXPERIMENTAL

These factors were investigated as follows:

1 — The degradation conditions:

A 1 % mixture of rivanol in polyethylene glycol 4000 was prepared (mixture A) by dissolving 1 g of rivanol in 9 ml of water, then by mixing the solution with 90 g of polyethylene 4000 on a water bath until a homogeneous mixture was obtained.

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The mixture was divided into 120 ampuls, 40 of these ampuls were sealed under nitrogen atmosphere, another 40 under carbon dioxide, the rest were unsealed. These three series of ampuls were divided into four parts and 10 ampuls of each series were heated at 105 °C, 10 ampuls of each series were exposed to UV, IR and day light respectively. The contents of the tubes were checked on thin layer plates in different time intervals, 0.1 n HCl : 96 % ethyl alcohol (1 : 1) was used as the solvent system. One unsealed ampul was exposed to day light about six months, this was used as reference studied and the following results were observed:

The latter gave four spots on thin layer plates and called mixture B. After development, thin layer plates of various samples were studied and the following restults were observed:

A — At zero time all samples showed one single yellow spot which was found to be identical to that of rivanol.

B — After an hour under various conditions, decomposition started and increased by time.

C — UV light exposure hastened the change of color in each series. After 3-5 hours of UV light exposure more than one spot were observed.

D — IR and day light exposures changed the colors in the ampuls to darker shades but not as much as UV light. In each case, after three hours more than one spot were seen on thin layer plates.

E — Under any conditions nitrogen atmosphere caused darker colors. The formation more then one spot started after an hour.

F — Carbon dioxide atmosphere under all conditions didn't change the colors. There was only one spot on each thin layer plate up to a week. After a week, degradation started in the ampuls that were exposed to UV and IR light and those which were heated. Even after a week no change was observed in those which were exposed to day light.

Table I shows these observations and gave the R_f values of the degradation products.

Conditions other than the above mentioned and which might hasten the degradation were also studied. A few drops of 1 % HCl

was added to mixture A and heated on a water bath about half an hour. At the end of the heating the acidic mixture showed two spots on thin layer plate with R_f values 0.69 (rivanol) and 0.55 (an orange colored spot).

The acidic mixture divided into four parts, one of them was heated to 105 °C, the others were exposed to UV, IR and day light respectively. After three hours, thin layer chromatographic control showed four spots. Calculated amounts of Cl_2, NH_3, H_2O_2, $KMnO_4$, KNO_3, $KClO_3$, and KIO_3 were added to mixture A respectively, in order to make their concentrations 1 %. All of these reagents caused darkening in the color of mixture A. On thin layer plates, even after an hour 2, 3 and 4 spots were observed. $KMnO_4$, H_2O_2 and Cl_2 were the most effective reagents on degradation.

Table II shows the results and gave the R_f values of degradation products.

2 — The study of the degradation products:

To obtain a reasonable quantity of degradation products, 10 g of rivanol and 90 g of polyethylene glycol 4000 were dissolved in water and since H_2O_2 left no residue was prefered and added to this mixture. The mixture was heated on a water bath about an hour. A black precipitate was formed in an orange solution. The precipitate was filtered, washed with cold water, dried and weighed, it was approximately 5 g. The black precipitate was partly soluble in hot water. This hot water soluble part was about 20 % of the precipitate and called (I). About 50 % of the precipitate was souble in hot 0.1 n HCl, this was called (II), the rest was soluble in 0.1 n NaOH (III). These various parts were checked on thin layer plates, it was found that they were single compounds. The black precipitate was also tried to be separated by column and by preparative thin layer and paper chromatographic methods, only minor amounts were obtained.

The UV and IR spectra of the degradation products were compared with that of rivanol. As it will be seen from Figure 1, the UV spectrum of rivanol has four maxima 435, 416, 387 and 290 mμ, whereas I, II, III have two maxima at 370 and 262 mμ ; 365 and 260 mμ ; 376 and 245 mμ respectively.

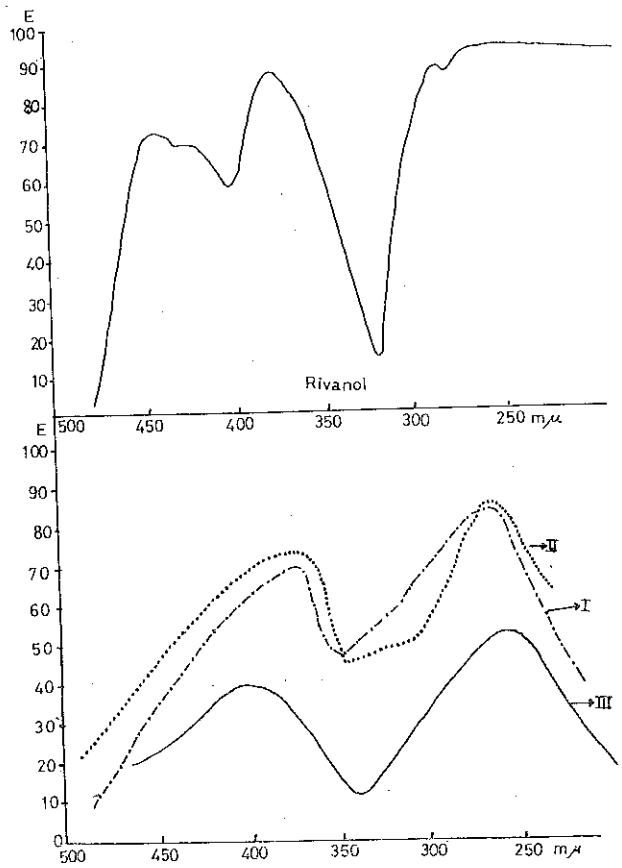

Fig. 1. UV spectra of rivanol and its degradation products (I, II, III).

In Figure 2 the IR spectrum of rivanol and its three degradation products were seen. As it is observed from the IR and UV curves there are important differences between rivanol and its degradation products. The degradation products are simpler compounds.

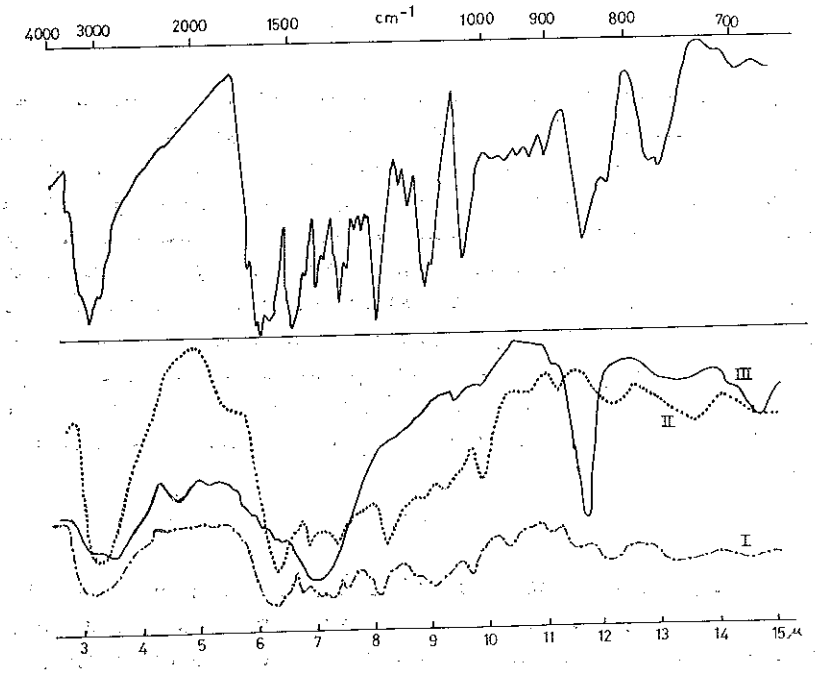

Fig. 2. IR spectra of rivanol and its degradation products (I, II, III).

3 — The comparison of the antibacterial activities of mixture A and degradation products:

The antibacterial activities of three degradation products were compared with that of mixture A. Stock solutions of degradation products and mixture A were prepared in 0.5 % concentrations. These were diluted to 1/1000; 1/5000; 1/10000; 1/15000; 1/20000; 1/25000; 1/30000; 1/40000; 1/50000; 1/60000.

Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, and *Pseudomonas aeruginosa* were used to control the antibacterial activity.

As it is seen from Table III, rivanol is active against *P. aeruginosa, S. aureus, K. pneumoniae* and *E. coli* at 1/10000, 1/15000, 1/30000 and 1/40000 dilutions respectively. Whereas three degradation products didn't show any activity against the above mentioned bacteria even in 1/1000 dilutions.

CONCLUSION

In this study it was observed that various conditions namely light (UV, IR, day light), heating, p_H, the presence of oxidizing agents, N_2 atmosphere increased the degradation of rivanol. Only carbon dioxide atmosphere either completely stops or greatly reduces the degradation. Since rivanol is used as an antibacterial agent, the antibacterial activity of rivanol and its degradation products were studied and it was found that the hot water, acid and alkaline soluble parts of the black precipitate has no activity on bacteria. IR and UV spectra of all degradation products proved that they are simpler compounds than rivanol. Since they have no antibacterial activity, further study on their structures found to be useless.

SUMMARY

The degradation conditions of rivanol was studied. The UV and IR spectra of degradation products were compared to that of rivanol. Their antibacterial activities were also compared to that of rivanol against four different bacteria, namely *S. aeruginosa*, *S. aureus*, *K. pneumoniae* and *E. coli*.

ÖZET

Rivanolün bozunma şartları incelendi. Bozunma ürünlerinin UV ve IR spektrumları rivanolünkilerle mukayese edildi. Ayrıca bozunma ürünlerinin bakterilere karşı olan etkileri dört değişik bakteri cinsinde, *S. aeruginosa*, *S. aureus*, *K. pneumoniae* ve *E. coli* de, rivanolünki ile kıyaslandı.

REFERENCES

1. Geçgil, Ş. *Eczacılık Bülteni*, **13**, 40 (1971).

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TABLE I. The Rf Values of the Degradation Products under Various Conditions.

Samples / Time	Day light exposure			UV light exposure			IR light exposure			Heating at 105°C		
	I	II	III	I	II	III	I	II	III	I	II	III
zero time	0.70	0.70	0.71	—	—	—	—	—	—	—	—	—
1 hour	0.70	0.69	0.70	0.69	0.70	0.70	0.69	0.70	0.72	0.70	0.70 0.55+ 0.45	0.70
3 hours	0.70	0.70 0.57+	0.70	0.70	0.69 0.55+	0,70	0.72	0.72 0.60+	0.70	0.70	0.70 0.55+ 0.45	0.70
24 hours	0.69	0.70 0.55+	0.70	0.70	0.70 0.55+ 0.45 0.20++	0.70	0.70	0.70 0.56+	0.70	0.72	0.70 0.55+ 0.46	0.70
2 days	0.70 0.56+	0.70 0.55+ 0.45	0.71	0.69 0.55+	0.70 0.55+ 0.45 0.20++	0.69	0.70 0.57+	0.69 0.56+ 0.47	0.70	0.71 0.55+	0.69 0.56+ 0.46 0.19++	0.69
5 days	0.69 0.55+ 0.45	0.71 0.55+ 0.45	0.69	0.69 0.55+ 0.45	0.71 0.56+ 0.46 0.22++	0.69	0.69 0.56+ 0.47	0.71 0.55+ 0.46 0.19+	0.71	0.71 0.56+ 0.46	0.69 0.55+ 0.47 0.20++	0.71
1 week	0.71 0.56+ 0.45	0.70 0.55+ 0.45 0.20++	0.71	0.71 0.55+ 0.45	0.72 0.55+ 0.46 0.20++	0.70 0.55+	0.70 0.55+ 0.45	0.71 0.56+ 0.45 0.20++	0.70 0.55+	0.70 0.55+ 0.46	0.69 0.55+ 0.45 0.20++	0.69 0.55+
6 months	0.69 0.57+ 0.45 0.20++	—	—	—	—	—	—	—				

I — Unsealed ampuls
II — Ampuls sealed under N_2
III — Ampuls sealed under CO_2

\+ Orange spots
++ Brown spots

TABLE II. The Rf Values of the Degradation Products

Sample / Time	zero time	1 hour				3		
		Day light	UV	IR	t°	Day light	UV	IR
1% HCl	0.70 0.55^{+}	0.70 0.55	0.69 0.56^{+}	0.71 0.56^{+}	0.72 0.55^{+}	0.70 0.56^{+} 0.40	0.70 0.56^{+} 0.40	0.70 0.56^{+} 0.40 0.20^{++}
1% Cl_2	0.69 0.55^{+}	0.70 0.55^{+} 0.45 0.20^{++}	0.69 0.56^{+} 0.45 0.20^{++}	0.70 0.55^{+} 0.45 0.20^{++}	0.70 0.55^{+} 0.45 0.20^{++}	0.69 0.56^{+} 0.45 0.20^{++}	0.71 0.55^{+} 0.45 0.20^{++}	0.70 0.55^{+} 0.45 0.20^{++}
1%NH_3	0.70 0.55^{+}	0.69 0.56^{+}	0.71 0.56^{+}	0.71 0.55^{+}	0.70 0.57^{+}	0.70 0.55^{+} 0.44	0.71 0.56^{+} 0.45	0.70 0.56^{+} 0.44
1% H_2O_2	0.70 0.56^{+}	0.72 0.56^{+} 0.45 0.20^{++}	0.71 0.55^{+} 0.44 0.19^{++}	0.70 0.56^{+} 0.45 0.20^{++}	0.72 0.57^{+} 0.46 0.21^{++}	0.71 0.55^{+} 0.40 0.20^{++}	0.70 0.55^{+} 0.40 0.19^{++}	0.69 0.54^{+} 0.44 0.19^{++}
1%$KMnO_4$	0.72 0.56^{+}	0.70 0.55^{+} 0.45 0.20^{++}	0.72 0.56^{+} 0.44 0.19^{++}	0.71 0.55^{+} 0.45 0.20^{++}	0.70 0.55^{+} 0.46 0.19^{++}	0.69 0.55^{+} 0.46 0.19^{++}	0.70 0.56^{+} 0.45 0.20^{++}	0.71 0.56^{+} 0.46 0.19^{++}
1% $K_2C_2O_7$	0.73 0.55^{+}	0.70 0.56^{+}	0.72 0.56^{+}	0.71 0.54^{+}	0.70 0.55^{+}	0.70 0.55^{+} 0.45	0.69 0.56^{+} 0.44	0.70 0.55^{+} 0.44
1%KNO_3	0.69 0.57^{+}	0.70 0.56^{+}	0.70 0.54^{+}	0.69 0.54^{+}	0.71 0.56^{+}	0.72 0.54^{+}	0.70 0.55^{+} 0.45	0.69 0.55^{+} 0.44
1%$KClO_3$	0.71 0.56^{+}	0.72 0.56^{+} 0.44	0.71 0.55^{+} 0.46	0.72 0.55^{+} 0.44	0.71 0.56^{+} 0.45	0.70 0.55^{+} 0.45 0.20^{++}	0.71 0.56^{+} 0.44 0.10^{++}	0.70 0.55^{+} 0.44 0.19^{++}
1%KIO_3	0.72 0.56^{+}	0.70 0.55^{+}	0.69 0.55^{+}	0.70 0.55^{+}	0.70 0.56^{+}	0.70 0.55^{+}	0.70 0.55^{+} 0.45	0.69 0.56^{+} 0.46

+ Orange spots ++ Brown spots

of Mixture A with Various Reagents.

hours	24 hours				72 hours			
t°	Day light	UV	IR	t°	Day light	UV	IR	t°
0.70 0.55+	0.70 0.55+ 0.42	0.71 0.57+ 0.44 0.20++	0.72 0.57+ 0.45 0.19++	0.71 0.55+ 0.46 0.20++	0.69 0.55+ 0.44 0.20++	0.69 0.55+ 0.45 0.20++	0.71 0.55+ 0.40 0.21++	0.70 0.55+ 0.40 0.20++
0.71 0.55+ 0.45 0.20++	0,69 0.55+ 0.45 0.20++	0.70 0.55+ 0.45 0.20++	0.69 0.55+ 0.45 0.20++	0.70 0.56+ 0.45 0.20++	0.71 0.55+ 0.45 0.20++	0.70 0.55+ 0.45 0.19++	0.69 0.56+ 0.45 0.20++	0.70 0.55+ 0.45 0.20++
0.69 0.55+ 0.44	0.70 0,56+ 0.45	0.71 0.55+ 0.45	0.69 0.55+ 0.44	0.70 0.56+ 0.44	0.70 0.55+ 0.40 0.20++	0.69 0.56+ 0.45 0.20++	0.70 0.55+ 0.45 0.20++	0.70 0.55+ 0.45 0.20++
0.70 0.55+ 0.45 0.20++	0.69 0.55+ 0.45 0,20++	0.71 0.56+ 0.45 0.19++	0.70 0.55+ 0.45 0.20++	0.72 0.56+ 0.44 0.20++	0,71 0.56+ 0.45 0.20++	0.70 0.54+ 0.44 0.19++	0.71 0.55+ 0.45 0.20++	0.70 0.56+ 0.45 0.19++
0.70 0.55+ 0.45 0.20++	0.72 0.56+ 0.46 0.19++	0.71 0.55+ 0.46 0.19++	0.70 0.56+ 0.45 0.21++	0.71 0.55+ 0.44 0.22++	0.70 0.55+ 0.45 0.20++	0.69 0.54+ 0.44 0.21++	0.70 0.55+ 0.44 0.21++	0.69 0.56+ 0.46 0.21++
0.71 0.56+ 0.44	0.72 0.55+ 0.44	0.71 0.56+ 0.46	0.72 0.55+ 0.44	0.72 0.56+ 0.45	0.71 0:55+ 0.44	0.69 0.54+ 0.45	0.70 0.54+ 0.45	0.70 0.54+ 0.45
0.69 0.54+ 0.45	0.70 0.55+ 0.44	0.71 0.55+ 0.45	0.70 0.56+ 0,45	0.71 0.55+ 0.40	0.72 0.55+ 0.45	0.71 0.56+ 0.46	0.72 0.54+ 0.44	0.71 0.55+ 0.45
0.69 0.55+ 0.45 0.20++	0.70 0.56+ 0.46 0.19++	0.72 0.56+ 0.46 0.22++	0.71 0.55+ 0.45 0.19++	0.72 0.56+ 0.46 0.20++	0.71 0.55+ 0.45 0.21++	0.70 0.56+ 0.44 0.20++	0.69 0.54+ 0.45 0.20++	0.70 0.54+ 0.44 0.19++
0.70 0.55+ 0.46	0.71 0.56+ 0.44	0.69 0.55+ 0.46 0.20++	0.70 0.55+ 0.46 0.19++	0.69 0.56+ 0.45 0.20++	0.70 0.55+ 0.46 0.19++	0.71 0.56+ 0,44 0.20++	0.70 0.55+ 0.46 0.19++	0.71 0.56+ 0.45 0.19++

TABLE III. The Comparison of the Antibacterial Activities of Mixture A and Degradation Products.

Rivanol concentration / Mixture A	1/1000	1/5000	1/10.000	1/15.000	1/20.000	1/25.000	1/30.000	1/40.000	1/50.000	1/60.000
S. aureus	—	—	—	—	+	+	+	+	+	+
K. pneumoniae	—	—	—	—	—	—	—	+	+	+
E. coli	—	—	—	—	—	—	—	—	+	+
P. aeruginosa	—	—	—	+	+	+	+	+	+	+

Products I, II, III										
S. aureus	+	+	+	+	+	+	+	+	+	+
K. pneumoniae	+	+	+	+	+	+	+	+	+	+
E. coli	+	+	+	+	+	+	+	+	+	+
P. aeruginosa	+	+	+	+	+	+	+	+	+	+