STNTHESIS OF DEHYDROACETIC ACID ISONICOTINYL HYDRAZONE POTASSIUM SALT. ITS *in vitro* ANTITUBERCULAR EFFECT ON A STRAIN OF VIRULENT HUMAN-TYPE TUBERCLE BACILLI.

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Introduction

In the preceding publications[1),2)], it was reported that the author synthesized dehydroacetic acid isonicotinyl hydrozone and its related compounds and especially determined an inhibitory concentration of the former against the growth of a strain of human-type tubercle bacilli *in vitro* and that the syntesized compound favourably affected the healing process of experimental progressive tuberculosis, as determined by macroscopic, microscopic, and bacteriological examinations. Keeping in mind EHRLICH's indisputable principle, "Corpora non agunst nisi liquida" the present writer newly synthesized its sodium salt soluble in water with all possible efforts and researched for the antitubercular activity, toxicity and physical and chemical properties, besides succeeded to administer it the tuberculous patients for a long term with clinically favourable responses but without recognizable side-effects. Moreover it was also found the newly synthesized compound histopatologically produced a pronounced therapeutic effect on the resected tuberculous pulmonary lesions[3)].

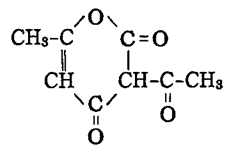
The elements of the first group in the periodic law table such as lithium, sodium, and potassium *etc.* resemble one another in chemical property. Above all, one of the characteristic properties of salts of sodium and potassium is high solubility in water. In addition in chemical reactions sodium and potassium are pressumed to react with other substances in the similar behaviour.

On the basis of the above mentioned considerations, the author synthesized dehydroacetic acid isonocotinyl hydrazone potassium salt.

In this report were presented preliminary observations concerning a synthesis process and antitubercular activity of the new chemical.

Synthesis of dehydroacetic acid isonicotinyl hydrazone potassium salt.

Dehydroacetic acid originated in GEUTER's discovery of the chemical synthesis between natrium acetic acid ethyl and carbonic gas in 1865. Since Dr. SEEVERS, professor of pharmacology at Michigan University introduced this substance as a useful antiseptic in 1951, in Japan it has been utilized only for food generally and recognized as having a broad-spectrum antiseptic. The constitutional formula of this material is as described under :



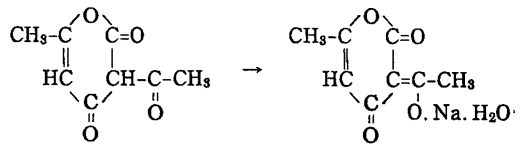
At first the meterial is suspended in distilled water in which K_2CO_3 solution is dropped in equivalent weight and agitated to get potassium salt. Then potassium salt water is concentrated under the low pressure and potassium salt is educed, filtered and washed with alcohol or acetone several times and dried well. Result of elementary analysis of the synthesized potassium salt is as shown in Table I and the theoretical value of it is given in Table II and both values show an approximate coincidence. Moreover the sodium salt can be written in the following equation.

Table I Results of elementary analysis of the systhesizad compound

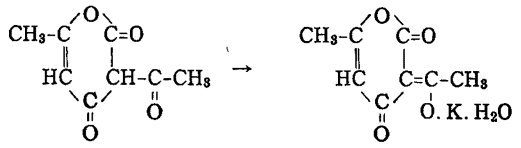
Carbon	Hydrogen	Oxygen	Kalium
42.91%	4.19%	35.69%	17.21%

Table II Theoretical values of elementary analysis of the compound

Carbon	Hydrogen	Oxygen	Kalium
42.85%	4.05%	35.67%	17.43%



Accordingly the chemical reaction of the potassium salt can be described in the following formula.



The product is tasteless, odourless and white-coloured powder which is, as expectedly, easily soluble in water.

As dehydroacetic acid potassium salt and isoniazid are forced to react in 90% alcohol by a return current system in equivalent weight, the both reac-

tion substances are caused to react with each other gradually and become yellow and transparent. In the reaction process, dehydroacetic acid isonicotinyl hydrazone potassium salt which is yellow cristalized solid precipitates. After the reaction is over, this is left for a while and then the desposits are taken out of alcohol washed out with heated alcohol and dried well.

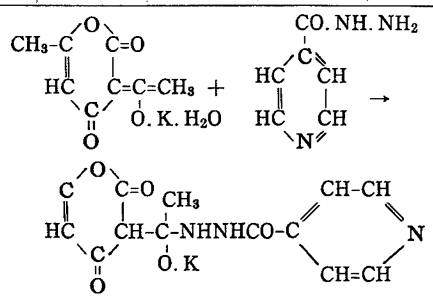
If isoniazid 1 g is reacted with dehydroacetic acid potassium salt 1.62 g in 90% alcohol 10 cc, the quantity obtained accounts to 2.1 g, and this is soluble in water and its melting point is at 255° C. The result of the elementary analysis of the synthesized chemical is as shown in Table III and that theoretical value of it is given Table IV and both values show an approximate coincidence. Its chemical formula is pressumed as described under.

Table III Results of elementary analysis of the synthesized compound

Carbon	Hydrogen	Oxygen	Nitrogen	Kalium
47.65%	4.09%	24.97%	11.98%	11.31%

Table IV Theoretical values of elementary analysis of the compound

Carbon	Hydrogen	Oxygen	Nitrogen	Kalium
48.97%	4.11%	23.29%	12.24%	11.39%



Antitubercular activity of the potassium salt *in vitro*.

The tubercle bacillus used for this test was the human virulent strain of *M. tuberculosis* (Kiyo H, isolated at the Research Institute of the Japan Anti-Tuberculosis Association). The culture medium was OGAWA's 1% KH_2PO_4 solid medium to which the newly synthesized compound was added in varying concentrations. 0.1 m of the bacillary suspension in concentration of 1 mg per ml was then into each medium. These media were incubated at 37° C for 6 weeks. The compound showed complete inhibition of the growth of the above-mentioned strain in concentration of 0.6 mcg per ml in OGAWA's 1% KH_2PO_4 solid medium for 6 weeks, as shown in Table V.

Table V Antituberculous activity *in vitro* of the compound

Concent. in mcg/ml \ Incubation	1 W	2 W	3 W	4 W	5 W	6 W
0.8	—	—	—	—	—	—
0.6	—	—	—	—	—	—
0.4	—	###	###	###	###	###
0.2	—	###	###	###	###	###

Inoculum : 28 day-old culture of Kiyo H_1, 0.1 mg
Incubation : 42 days at 37° C

Discussion

Since the advent of isoniazid into the treatment of tuberculosis, it is well known that isoniazid has produced an excellent antitubercular activity on tuberculosis. Many kinds of its derivates have been synthesized at home and abroad so that they may be given for a long term with less side-effects and more favourable responses upon tuberculous patients. The author synthesized a tuberculostatic compound by a chemical reaction between dehydroacetic acid sodium salt with a broad antibacterial spectrum and isoniazid and went into details of its antitubercular activity of the compound about the therapeutic effect upon clinical tuberculosis.

From the standpoint of the periodic law of the elements, dehydroacetic acid potassium salt and dehydroacetic acid isonicotinyl hydrazone potassium salt have been synthesized and especially the latter has been researched for its antitubercular activity against the growth of virulent human type tubercle bacilli *in vitro*. It is pressumed that dehydroacetic acid potassium salt has a broad antibacterial spectrum as dehydroacetic acid sodium salt does. But it will require future study to decide whether or not dehydroacetic acid isonicotinyl hydrazone potassium salt by itself (without decomposition) shows antibacterial activity on other bacteria than tubercle bacilli.

As to the determination of more correct constitutional formula of dehydroacetic acid isonocotinyl hydrazone potassium salt, including its sodium salt, further investigations should be required. Be that as it may, it is pressumed that the compound will produce therapeutic effect upon clinical tuberculosis.

Conclusion

The author synthesized dehydroacetic acid potassium salt and dehydroacetic acid isonocotinyl hydrazone potassium salt, besides the minimal inhibitory concentration of the latter against the growth of virulent human type tubercle bacilli was determined.

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References

1) KUNIGOSHI, U. . Annual report of the Japanese Association for Tuberculosis. 2 : 61, March, 1957. 2) KUNIGOSHI, U. : Chemotherapy 6 (5) : 336, 1958. 3) KUNIGOSHI, U. *et al.* : To be published. 4) Jap. P. : 30～2032.