SYNTHESIS OF DEHYDROACETIC ACID ISONICOTINYL HYDRAZONE SODIUM-SALT. ITS ANTITUBERCULAR EFFECT ON CLINICAL TUBERCULOSIS

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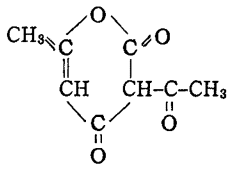
(Received March 13, 1958)

Introduction

By a chemical reaction between dehydroacetic acid and isoniazid, the author synthesised dehydroacetic acid isonicotinyl hydrazone and its hydrochloride salt and especially decided an inhibitory concentration of the former against the growth of the Kiyo H_1 strain (isolated at the research institute of Japan Antituberculosis Association) of *M. tuberculosis in vitro* and went into details of its antitubercular activity of the synthesised compound about the therapeutic effect upon the experimental tuberculosis from the microscopic, bacteriological and pathological point of view. Keeping in mind the indisputable principle "Corpora non agunst nisi liquida" of the originator of chemotherapy, EHRLICH, the present writer had exerted all possible effort to synthesise its sodium-salt, and so researched for the antitubercular activity, toxicity and physical and chemical properties of the new material, besides administered it to the tuberculous patients for a long term and observed how it worked. Nowadays in chemotherapy for the tuberculous patients, two or three chemotherapeutics are used in combination, but in this clinical research the new compound is not used with any other medicine, because this is a new one and the chief aim of this search is to know the therapeutic effect by itself.

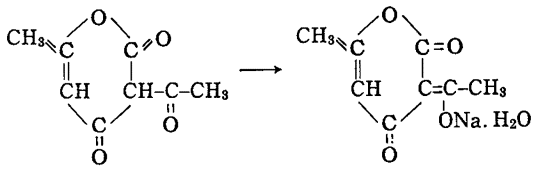
Synthesis of dehydroacetic acid isonicotinyl hydrazone sodium salt. Its chemical and physical properties.

Dehydroacetic acid was initially synthesised by GEUTER in 1865. In Japan dehydroacetic acid is used only for antiseptic for food generally and it is distinguished by the broad antibacterial spectrum, The constitutional formula of this material is as described under.



At first the material is suspended in distilled water in which 10% Na_2Co_3 solution is dropped in equivalent weight and agitated to get sodium salt. Then sodium salt water is concentrated under the low pressure and sodium salt is educed, filtered, washed with alcohol or acetone several times and dried well.

In another way when dehydroacetic acid is dissolved in benzol or toluol, and equivalent Na_2Co_3 is dropped, agitated as stated above, sodium salt is generated and educed in precipitation. Filtration, washing, and drying followed as well as above mentioned. The reaction formula is as follows.



The product is tastless, odorless and white colored crystal powder is soluble not only in water but in fat like olive oil, and moreover it suggests the possibility of solvency in caseous foci.

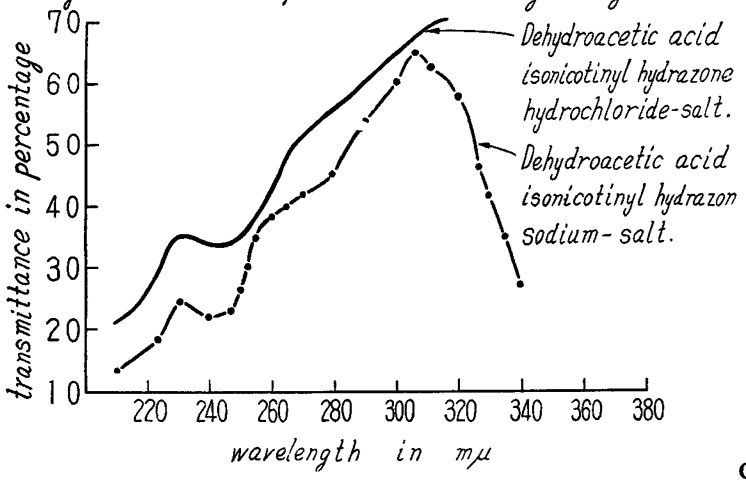
It inhibits the growth of virulent human type tubercle bacilli in 1,500 mcg per ml in Ogawa's 1 % KH_2PO_4 medium and has the broad antibacterial spectrum as the former substance does.

The extra-violet ray analysis was performed on dehydroacetic acid isonicotinyl hydrazone hydrochloride-salt and its sodium salt, each of which is dissolved in water adjusted to pH 6.2. The result of it is as shown in Figure 1. From this it is known that there is an absorption spectrum close by the absorption-spectrum 299 mμ peculiar to dehydroacetic acid. It is assumed that there is a molecular structure of dehydroacetic acid in molecular structure of dehydroacetic acid sodium salt. Consequently the way of making the material react with isoniazid is taken in order to synthesise sodium salt.

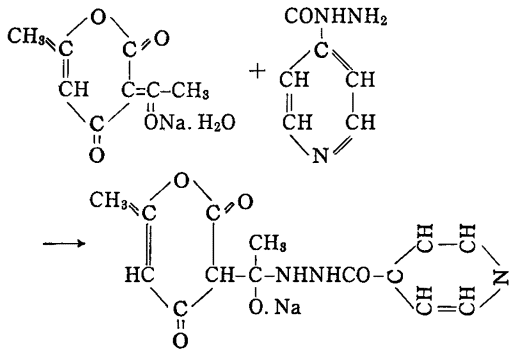
As dehydroacetic acid sodium salt ann isoniazid are forced to react in 90% alcohol by a return current system in equivalent weight, both reaction substances are caused to react with each other gradually and become yellow and transparent and then dehydroacetic acid isonicotinyl hydrazone sodium salt which is yellow crystal is educed.

If isoniazid 3.0 g is reacted with dehydroacetic acid sodium salt 4.56 g in 90% alcohol 30 cc, dehydroacetic acid isonicotinyl hydrazone sodium-salt is abundantly educed. After the reaction is over, it

Figure 1. Result of extra-violet ray analysis



is taken out from alcohol and washed with alcohol and dried. The quantity obtained is 6.2 g, its melting point 247°C, and its ash (as Na_2O) 8.97% which is nearly equal to the theoretical value 9.46 %. This suggests that the new compound is pure. The solubility in water (20°C) is 0.51 g. Its chemical formula is as described under.



Stability against acid of the compound is kept even at pH 4.0 and that against alkali is not unchanged in 40% NAOH solution.

The antitubercular activity of the substance *in vitro*. The substance produced complete inhibition of the growth of the human type strain tubercle bacilli in concentration of 0.6 mcg per ml in Ogawa's 1% KH_2PO_4 solid medium[2], as shown in Table 1.

Table 1. Antitubercular Activity *in vitro* of the Compound.

Inoculum: 30-day-old culture of Kiyo H_1, 0.1 mg.
Incubation : 42 days at 37°C.

Concent. in mcg/ml \ Incubation	1 W	2 W	3 W	4 W	5 W	6 W
1.6	—	—	—	—	—	—
0.8	—	—	—	—	—	—
0.6	—	—	—	—	—	—
0.4	—	###	###	###	###	###
0.2	—	###	###	###	###	###
0.1	—	###	###	###	###	###

Note ; ###, bacilli grow all over culture medium.
—, no bacilli grow.

Acute toxicity test BEHRESN-KÖRBER's test of acute toxicity showed a very low value as compared with that of isoniazid, as will be described under. Isoniazid : mouse, LD_{50}=200 mg/kg per os. Dehydroacetic acid isonicotinyl hydrazone sodium salt : Mouse, LD_{50}= 860 mg/kg, per os.

Clinical observations

Patients : The five patients chosen for study were all adults with pulmonary tuberculosis and had not received any other antitubercular agents. The four patients were dischanging tubercle bacilli which were susceptible to streptomycin, and para-aminosalicylic acid and were incompletely resistant to 0.1 mcg per of OGAWA's 3% KH_2PO_4 solid medium.

Dosage regiment For long-term administration, the drug was given orally in a total daily dose of 1 g per person, divided into three doses after meals.

Administration period Six months.

Tubercle bacilli detection in sputum : Unconcentrated specimens of sputum were examined once monthly by the stained smear technique (ZIEL-GABBET's method) and culture one (OGAWA's method). Urinalysis : Albumin, sugar, urobilinogen, urobilin, and sediment were examined once monthly.

Test of hepatic function : B. S. P. test was performed once monthly.

Hematologic study : Erythrocyte and leukocyte counts, differential leukocyte counts, determinations of hemoglobin and sedimentation rate were made once monthly.

Roentgenograms : Chest films including tomographs were taken at onset of therapy and every three months there after for evaluation of the effectiveness of the drug.

Results

In all the patients the effectiveness of the drug was observed. The roentgenograms indispensable for the evaluation of drug-effectiveness showed improvement, with cavities somewhat smaller.

Temperature : All patients but one were afebrile at the onset of therapy. In the one patient, the temperature returned to normal in about one month. In the others, a temperature elevation was not

observed.

Weight All patients had the weight gains. It is essential to note that, although steadily sought, abnormal accumlations of body fluids were not found. One patient has gained as many as 15 pound at the end of the experiment.

Appetite : Generally speaking, appetites are not susceptible of objective estimation but have been uniformly remarkable in the present series.

Cough and expectoration : Relief from cough and expectoration was noted with regularity.

Erythrocyte sedimentation rates : They were essentially unaffected in the cases with the normal range, in some instances with an abnormal range, a tendency toward minor elevation was noted.

Tubercle bacillus in sputum : With exception of a case without tubercle bacilli on smear and culture, three cases were negative for tubercle bacilli on both smear and culture and one case was smear-negative and culture-positive although they had been positive on smear at the onset of therapy.

The tubercle bacilli stayed less than 0.1 mcg of soniazid in drug-resistance test.

Findings of chest films In all instances, comparison of interval with pre-therapy showed reduction in cavity size and resorption of exudative disease present.

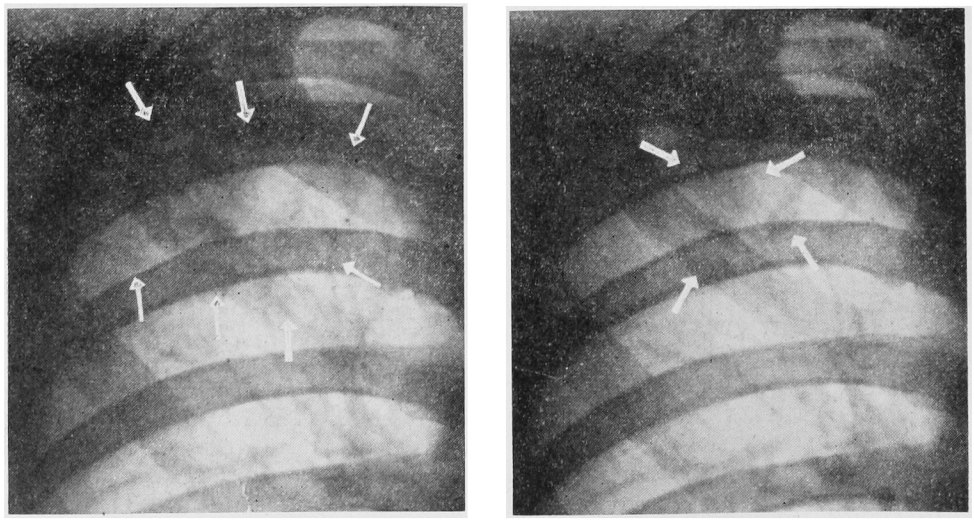
Case reports

Case 1. (J. S.). This 23-year-old student was admitted to this hospital on June 28, 1957, with caseous tuberculosis in the right infraclavicular region, the tomographs revealing no apparent cavities. He had ever been suffering from exsudative pleurisy. One month after the hospitalization, the tuberculous lesion and slight cough and expectration persisted. Therapy with the compound was started on July 27, 1957. Three months after therapy, the shadow was already decreased, the tomographs showing improvement. His cough and expectration were entirely gone.

Case 2. (G. T.). The 31-year-old male was admitted to the hospital with bilateral caseous-pneumonic disease in infraclavicular lesions, cavities being especially in the left side, on June 3, 1957. He had been attacked by pneumonia and cough and expectoration were moderately observed. The sputum was positive for tubercle bacilli on all examinations. Therapy was started on June 6, 1957. Three months after therapy the sputum was negative for tubercle bacilli on the examinations. The clinical improvement was continued and expectorations were entirely disappeared. A rontgenogram after 6 months showed a disappearance of the right-sided shadow and decrease in cavity in size.

Case 3. (K. S.). The thirty-one-year male was admitted to the hospital with fibrotic exsudative tuberculosis on May 6, 1957. He had had often common cold, expectoration and weight loss for six months. The sputum was positive for tubercle bacilli in all examinations. After three months therapy, cough and expectroation were markedly decreased in which tubercle bacilli were not found. At the end of therapy, a roentgenogram showed marked improvement, with the cavity markedly decreased in size.

Case 4. (T. W.). The 32-year-old male was admitted to the hospital with right-sided cavitary caseous tuberculosis. He had no complaints, subjectively. The cough and sputum were slightly present,

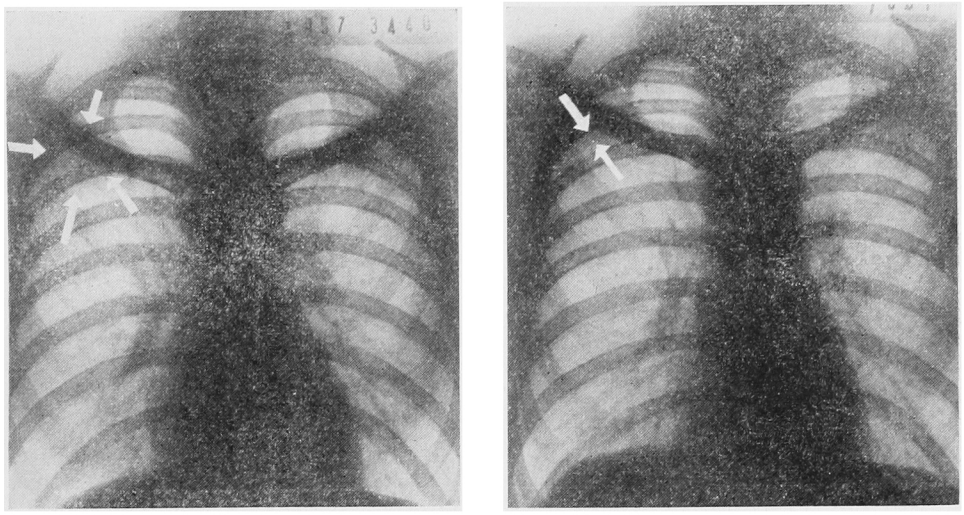


Case 1.

Left. (J. S.) Roentgenogram before therapy showing infiltration here and there in upper lung field.
Right. Roentgenogram after therapy showing moderate resorption of lesions.

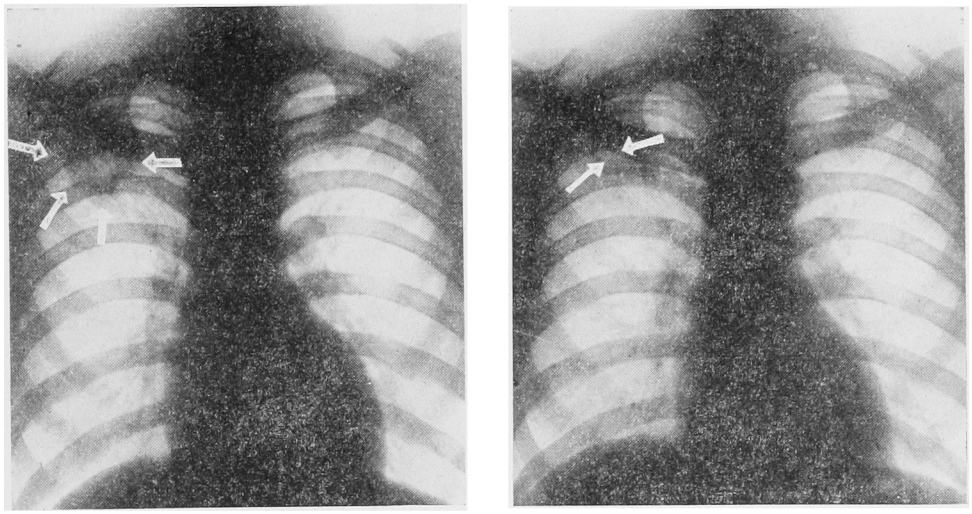
but the sputum was proved to be positive for tubercle bacilli on smear. Therapy was begun on July 11, 1957. Three months after therapy, cough and sputum was entirely diminished, in which tubercle bacilli were not found on smear and culture. A roentgenogram showed diminution of infiltration, tomograph showing marked decrease of cavity in size.

Case 5. (M. S.). This 28-year-old female first suffered from symptoms of tuberculosis such as cough, expectoration, blood-tinged sputum and night sweating, chest pain and loss of weight in October 1956. In July, 1957, an excerbation occurred and she was admitted to the hospital on July 9, 1957. On admission she was classified as having far advanced caseous pneumonic tuberculosis with positive sputum on smear and culture. Fever ranged from 101°F to 102°F. A cavity was radio photogra-



Case 2.

Left (G. T.) Roentgenogram before onset of therapy showing infiltrate in both infraclavicular regions.
Right. Roentgenogram after therapy showing disappearance of right infiltrate and slight reduction of left lesion.

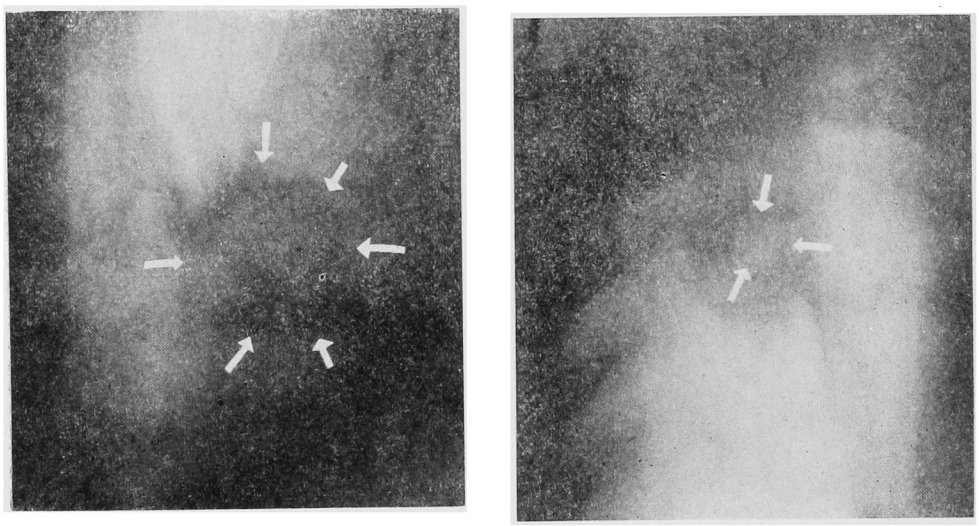


Case 3.

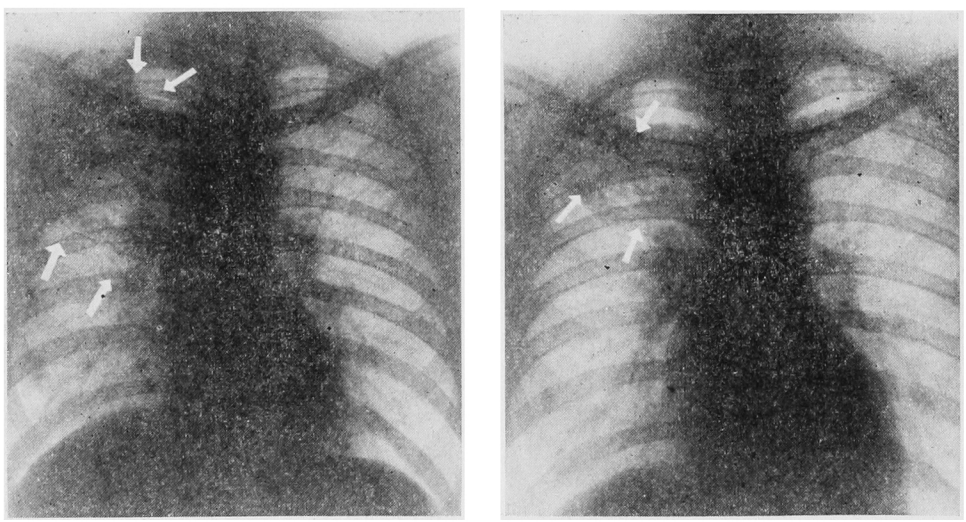
Left (T. W.) Roentgenogram before onset of therapy showing large cavity surrounded by exsudation in right infraclavicular region.
Right. Roentgenogram after therapy showing marked reduction of the cavity in size and slight reduction of the infiltration.

phically proved. At the time of institution of therapy she was of subikterus in skin. Therapy of the compound was begun on July 23, 1957. At the end of study a roentgenogram showed marked resorption of exsudative disease. The sputum was negative for acid-fast bacili, but the culture revealed tubercle bacilli remained to be resistant to isoniazid in the range of 0.1 mcg per ml in OGAWA's solid medium. She had gained 17 pounds and the temperature became normal after two months. Blood sedimentation rate fell to 40 mm from 140 mm in an hour at the end of study.

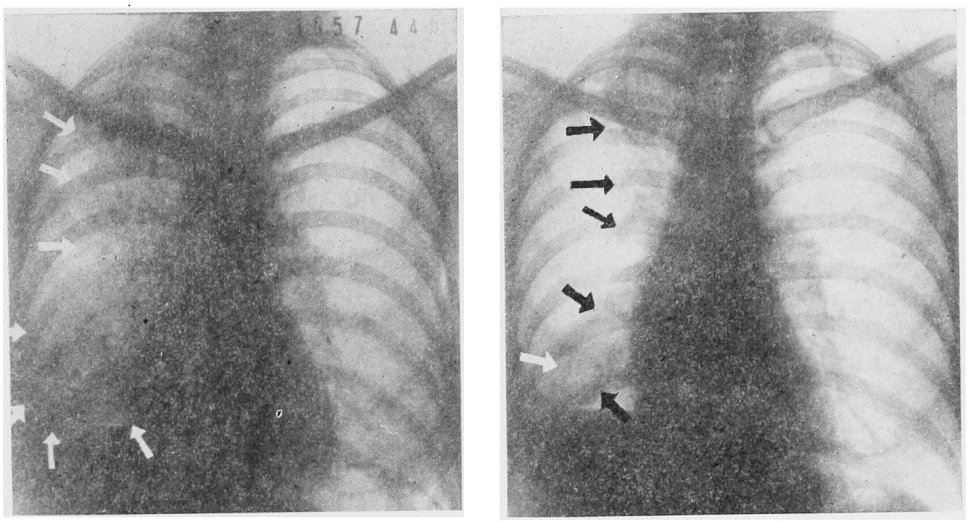
Side-effects: As a result of urinalysis and B.S.P. test, hepatic hypofunction was not observed in all instances. Hematologic study showed no significant changes, and no allergic response was noted clinically, nor were eosinophilia, hemorrhagic tendency and central nervous system disturbance found through the experiment.



Case 3.
Left. (T.W.) Pretherapeutic tomograph showing large cavity.
Right. Posttherapeutic tomograph showing marked reduction in the cavity size.



Case 4.
Left. (K.S.) Roentgenogram before onset of therapy showing extensive exsudate within which cavity is visible in right upper lung field.
Right. Roentgenogram after therapy showing marked reduction of the exsudate and the cavity in size.



Case 5.

Left. (M. S.) Roentgenogram before onset of therapy showing extensive exsudative infiltrate with large cavity in the lower lung field.

Right. Roentgenogram after therapy showing marked resorption of the exsudative disease and slight reduction in cavity size.

Discussion

Since the practical effectiveness of isoniazid upon tuberculosis had come to be widely recognized, various kinds of its derivates have been synthesised at home and abroad, in order to be administered for a long term with less side effects and more favorable responses on tuberculosis. With some hope to add my share of contribution to this trend, the author has made a chemical synthesis between isoniazid, an excellent antitubercular agent, and dehydroacetic acid sodium-salt with a broad antibacterial spectrum, producing dehydroacetic acid isonicotinyl hydrazone sodium-salt. It is interesting that although in one case, the tubercle bacilli stayed in resistance of 0.1 mcg per ml of isoniazid, suggesting peculiarity of the new synthesised compound. Mereorer, the similar results will be probably obtained when K_2CO_3 is used in stead of Na_2CO_3 from the standpoint of the periodic law of the elements.

Conclusion

Though in this limited experience, the compound alone was to be proved an effective agent in the long-term chemotherapy on clinical tuberculosis, withot recognizable side-effects. The main purpose of this present report may adequately be concluded by paraphsaing the author's statement. Primary aim of this report is not to impart knowledge but to increase understanding in the synthesis of chemotherapeutics on which future studies may advance.

Acknowledgment

The author wishes to express gratitude to Dr. TASAKA, professor of TASAKA clinic of internal medicine, School of medicine, Tokyo University, and to Dr SHIMAMURA, president of our hospital for their review of this report.

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