49. Research on Xanthurenic Acid. XVI

Action of 4-Hydroxy-8-Methoxy-quinoline-2-Carboxylic Acid as the Inhibiting Agent with regard to the Diabetogenic Property of Xanthurenic Acid

By Yahito KOTAKE and Muraji KATÔ

Biochemistry Department of Wakayama Medical College

(Comm. by Y. KOTAKE, M.J.A., March 12, 1956)

Introduction. In a previous report, xanthurenic acid, an abnormal metabolite of tryptophan, was pointed out by Yahito Kotake and Toshiro Inada¹⁾ to be a diabetogenic substance. Oxine, likewise, was formerly detected by Kôzo Okamoto²⁾ as another experimental diabetogenic substance. On the basis of these two discoveries, it is possible to consider that the presence of free OH radical in the 8 position of quinoline ring is an indispensable condition for quinoline compound to be diabetogenic in its property.

Yahito Kotake et al. in their experiments on diabetic symptoms presented in animals by means of synthetic xanthurenic acid administration, always laid stress on the purity of substances. In the last process of hydrolysis of 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid ethylester when either the original Musajo's method³⁾ or the reformed Musajo's method was employed to synthesize xanthurenic acid, they, therefore, repeatedly recrystallized xanthurenic acid which they had obtained as a result of demethylation with HI.

Besides, in order to make sure the purity of the synthetic crystallized xanthurenic acid thus obtained, it was subjected to the rate of Fe^{+++} colour reaction and its paper-chromatographical test by adopting as its control the pure xanthurenic acid isolated from the urine of albino rats, and tried to confirm especially 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid was not mixed with it. It is not seldom that this substance was found in co-existence with synthetic xanthurenic acid as impurity. And in such a case, xanthurenic acid was often found by Yahito Kotake et al. to be incapable of developing any diabetic symptoms.

This fact has further led the authors to a possible conclusion

that when OH radical in the 8 position of xanthurenic acid is substituted by methoxy radical, xanthurenic acid not only loses its diabetogenic property, but also inhibits such a diabetogenic action. Hence the experiments using crystals of synthetic xanthurenic acid, 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid ethylester and its free acid were tried. The satisfying results thereof are here reported as follows.

Experimental. (1) Materials and procedure: Albino rats fed more than a fortnight on a casein diet (casein 22%, McCollum salt mixture 6%, agar-agar 3%, yeast 2%, butter 10%, sugar 5%, and starch 52%), and weighing around 200 g.

Xanthurenic acid (m.p. 296.5°C) and 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid ethylester (m.p. 109°C) were synthesized in accordance with the reformed Musajo's method. Their synthetic processes are shown below.

As stated before, what is of utmost importance concerning synthesized xanthurenic acid is the question of its purity. Xanthurenic acid is obtained by demethylation of 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid ethylester, but the treatment on this occasion is very difficult. Unless extreme care is taken, a small quantity of 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid is often mixed with xanthurenic acid. The impurity contained in it, in this case, may possibly be considered to be not ester but carboxylic acid (m.p. 249–250°C). Prior to the experiment, therefore, a strict examination of the purity of xanthurenic acid, especially by means of paper-chromatography, was required. Furthermore, when *n*-butanol:acetic acid:water=4:1:1 was used as developer, the *R_f* value of xanthurenic acid was found to be around 0.48, while that of 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid around 0.75. As for the fluorescent reactions, the former presented pale blackish colour, while the latter brilliant blue and did not develop colour reactions of xanthurenic acid such as Pauly's diazo, Ehrlich's diazo, and Fe⁺⁺⁺, etc.

(2) Blood sugar measurement. The measurement was conducted in accordance with King-Garner's⁴⁾ method. (In this method normal value of blood sugar is about 70 mg/dl.)

Results and Discussion. Action of 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid ethylester and its free acid as the inhibiting agent with regard to the diabetogenic property of xanthurenic acid.

A group of albino rats was intraperitoneally injected with 200 mg of xanthurenic acid per kg as control animals, another

group of animals was injected with 100 mg, 50 mg, or 20 mg of 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid ethylester per kg and ten minutes later, re-injected intraperitoneally with 200 mg of xanthurenic acid per kg this time. The changes of the blood sugar amount measured then in terms of time are shown in Figs. 1, 2, and 3.

Fig. 1. Tested animals: Xanthurenic acid 200 mg/kg and 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid ethylester 100 mg/kg inj.

Fig. 2. Tested animals: Xanthurenic acid 200 mg/kg and 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid ethylester 50 mg/kg inj.

Control animal

Tested animals

Fig. 3. Tested animals: Xanthurenic acid 200 mg/kg and 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid ethylester 20 mg/kg inj.

Control animal

Fig. 4. Tested animals: Xanthurenic acid 200 mg/kg and 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid 50 mg/kg inj.

Tested animals

In each of these experimented animals 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid ethylester was found to be capable of inhibiting the diabetogenic property of xanthurenic acid in a remarkable degree. No such post-injection changes as the initial hyper-

glycemia, hypoglycemia, and continued hyperglycemia observable in the control animals could in any way be detected, and it was fully proved that the co-existence of this substance in an amount of 1/10 or even less of xanthurenic acid injected could completely inhibit the diabetogenic property of the latter.

As the impurity contained in synthetic xanthurenic acid may possibly be considered to be not ethylester but free 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid, the experiment using this free acid was tried. In this case this free acid could also inhibit the diabetogenic property of xanthurenic acid in the same degree of ethylester (Fig. 4).

In view of this finding, it is now safe to conclude that for any of the substances with quinoline ring to have its diabetogenic property, free OH radical in the 8 position of quinoline ring is utterly indispensable and that if this OH radical is substituted by methoxy radical, the substance not only loses its diabetogenic property, but also inhibits the diabetogenic action of xanthurenic acid itself. This fact further reveals it to be an indispensable condition that in an experiment for diabetogenic properties using synthetic xanthurenic acid care should be taken that 4-hydroxy-8-methoxy-quinoline-2-carboxylic acid which can so easily be contained is completely eliminated before its actual employment.

In conclusion, we heartily thank Prof. Yashiro Kotake for his pertinent advice and encouragement, and also heartily thank Prof. Takeo Sakan for his advice to synthesis.

References

- 1) Kotake, Yahito, and Inada, T.: Proc. Japan Acad., **28**, 65 (1952).
- 2) Okamoto, K.: Acta scholae medicales universitatis in Kioto, **27**, 43 (1949).
- 3) Musajo, L., and Minchilli, M.: Ber. deutsch. Chem. Ges., **74**, 1839 (1941).
- 4) King, E. J., and Garner, R. J.: J. Clin. Path., **1**, 30 (1947).