United States Patent Office 3,836,639 Patented Sept. 17, 1974

3,836,639

USE OF AROMATIC GLYOXALS, THEIR HYDRATES AND BISULFITE ADDITION SALTS IN REDUCING BLOOD PLATELET AGGREGATION

Daniel M. Teler, Devon, Charles J. Guinosso, King of Prussia, Stanley C. Bell, Penn Valley, and Richard L. Fenichel, Wyncote, Pa., assignors to American Home Products Corporation, New York, N.Y.

No Drawing. Filed Sept. 19, 1973, Ser. No. 398,898
Int. Cl. A61k 17/00, 27/00
U.S. Cl. 424—101 9 Claims

ABSTRACT OF THE DISCLOSURE

Certain aromatic glyoxal derivatives, their hydrates and bisulfite addition salts are useful anti-blood platelet aggregation agents *in vitro* and *in vivo*.

BACKGROUND OF THE INVENTION

The phenyl glyoxals of this invention are known compounds, with the exception of 2,6-dichlorophenylglyoxal, the preparation of which is presented *infra*. J. Am. Chem. Soc., *79*, 1687 (1957) and Biochem. Z., *279*, 459 (1935). Similarly, the bisulfite addition products of the compounds used in the instant invention are known with the exception of the *p*-tolyl glyoxal derivative.

DESCRIPTION OF THE INVENTION

In accordance with this invention there is provided a new use for various aromatic glyoxal derivatives which comprises a process for inhibiting blood platelet aggregation by adding to said platelets *in vitro* or *in vivo* an effective amount of a compound selected from the group consisting of phenyl glyoxal, *p*-tolyl glyoxal, *p*-methoxyphenyl glyoxal, *p*-chlorophenyl glyoxal, 2,6-dichlorophenyl glyoxal, and the hydrates and bisulfite addition salts thereof. The effective amount of the active compounds employed *in vitro* may be readily determined from the data provided *infra*. For *in vivo* administration the effective amount is a prophylactic amount based upon the requirement of the individual patient.

In the prophylactic treatment of patients subject to potential blood platelet aggregation such as in the case of patients suffering from diabetes, hypertension, or presenting a history of myocardial infarction or in the case of cerebral vascular accidents etc., the anti-platelet aggregation agents prevent the breakdown of blood platelets and the possible consequential thrombosis resulting from such breakdown. The compounds employed in this invention may be administered orally or intramuscularly, in the case of an emergency, in a daily dose up to 600 milligrams per a 70 kilogram patient. The compounds used in the present invention may be administered in multiple dose form up to and including 600 milligrams per day, as prescribed by a physician, taking into consideration the age, general physical condition, weight, and medical history of the patient being treated. As such, the process of this invention is useful in the treatment of the arteriosclerotic process which may begin in a thrombus formation via blood platelet aggregation or clotting.

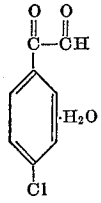
The *in vitro* activity of the compounds employed in the process of this invention as inhibitors of blood platelet aggregation was evaluated, essentially following the procedure of Born, G. V. R. and Cross, M. J., J. Physiol., *168*, 178–195 (1963). The *in vitro* studies were made on platelet rich plasma obtained from normal fasted male

rats. Adenosine diphosphate was added to the platelet rich plasma in an amount predetermined to maximize platelet aggregation. A curve of percent light transmission at 610 millimicrons was plotted for a 7 minute period. The compounds being tested were incorporated in identical formulations and the concentration affecting 50 percent inhibition of the adenosine diphosphate activity was determined by comparison with a standard curve. The compounds employed in the process of this invention inhibited blood platelet aggregation at concentrations as low as about 0.03 millimolar, while increased activity occurred with increased concentration.

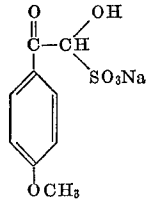
The *in vitro* studies in accordance with the preceding paragraph were repeated employing, in lieu of platelet rich plasma obtained from normal fasted rats, human platelet rich plasma. The compounds employed in the process of this invention inhibited platelet aggregation in human platelet rich plasma at concentrations as low as about 0.5 millimolar, with increased activity occurring with increase concentration.

For *in vivo* activity, the compounds were tested for their capacity to inhibit adenosine diphosphate (ADP) induced reduction of circulating blood platelets in control and treated groups of male Sprague Dawley rats. A control cardiac blood sample was taken and each compound tested was given to the experimental group at a starting dose of 100 mg./kg. or lower depending on the nature of the compound under test. After 30 minutes 15 mg./kg. of ADP was injected into the leg vein. Cardiac blood samples were taken at 20, 40 and 60 seconds. The control group was given only ADP. Platelet counts were made on all blood samples with a Coulter Counter, the results plotted, and the percentage of inhibition determined. Active compounds were run at lower concentrations, and the results are expressed as the lowest dose showing significant inhibition of the ADP effect.

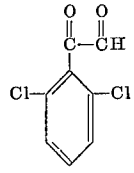
The biological activity of the glyoxal derivatives employed in the process of this invention are presented in the following table in which the concentration of the specific compound employed which gives a 50 percent inhibition of adenosine diphosphate induced blood platelet aggregation is presented. The first number appearing is that obtained from studies with platelet rich plasma obtained from normal fasted male rats, the second number presented is the data obtained from treatment of human platelet rich plasma, and the third set of data represents the results of *in vivo* experiments expressed in terms of milligrams per kilogram host body weight. It should be noted that the indication of "slight activity" or "no significant activity" is not an indication of absence of activity in human blood platelet aggregation inhibition. In practice, the species difference between the human and the standard test animal—the rat—is such that the rats blood platelets are considerably more resistant *in vivo* to adenosine diphosphate aggregation than are human platelets. Hence, the purpose for performing the more stringent tests on live rats was to establish additional information relative to the degree of effective activity of the compounds being tested, rather than to establish the fact of activity in the human, which is shown by the studies on human platelet rich plasma. Hence, the *in vivo* data must be interpreted from the standpoint of demonstrating the most active member(s) of a family of compounds being tested, rather than as a definitive test directly correlated with the human upon which the absence of activity of a compound can be predicated.



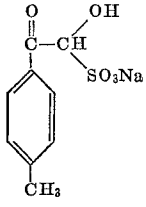
(3.3×10⁻⁵M)
(5×10⁻⁴M)
25 mg./kg.



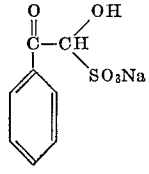
(1.4×10⁻⁴M)
(1.25×10⁻³M)
25 mg./kg.



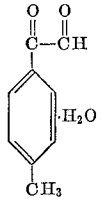
(1.2×10⁻⁴M)
(7.6×10⁻⁴M)
50 mg./kg.—no significant activity



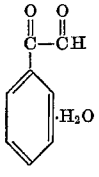
(1.9×10⁻⁴M)
(7.1×10⁻⁴M)
25 mg./kg.



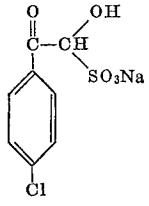
(6.2×10⁻⁴M)
(1.2×10⁻³M)
50 mg./kg.—slight activity



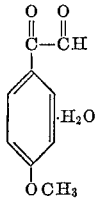
(2.2×10⁻⁴M)
(5.0×10⁻⁴M)
50 mg./kg.—no significant activity



(2.3×10⁻⁴M)
(5.8×10⁻³M)
25 mg./kg.



(2.3×10⁻⁴M)
(4.9×10⁻⁴M)
50 mg./kg.—no significant activity



(3.1×10⁻⁴M)
(2.4×10⁻⁴M)

It is to be understood the process for preventing blood platelet aggregation presented in this application embraces the use of the disclosed compounds in treating human patients on a prophylactic basis where the tendency for blood platelet aggregation has been initiated by a disease, or the prevention of platelet aggregation in storage for subsequent use in the treatment of patients suffering from leukemia, thrombocytopenia, and similar maladies. As an anti-platelet aggregation agent, the glyoxal derivatives employed in this invention preserve blood platelets, preventing stickiness or aggregation, for subsequent administration to the patient.

The anti-platelet aggregation agents of this invention may be used by themselves for treatment of platelets prior to storage, or conventional formulations incorporating the active compounds may be formed for ease in dispensation, and in the case of formulations for *in vivo* treatment of warm-blooded animals, conventional adjuvants known to the pharmaceutical chemist may be added to the active ingredient in appropriate measure. For example, tablets may be prepared to contain approximately 80 parts sucrose, 13 parts starch, 6 parts magnesium stearate and dose multiples of active ingredient ranging from ca. 50, 100, 200 to 250 parts. Likewise, injectible solutions are prepared by dissolving the active compound in the desired concentration of water or alcoholic vehicle with or without buffer, stabilizer, etc.

The following preparative procedures for producing the glyoxal derivatives employed in the process of this invention are presented for the purpose of illustrating preparative techniques which may be used in the production of the known compounds employed in this invention as well as the novel derivatives thereof.

EXAMPLE 1

2,6-Dichlorophenyl glyoxal

A solution of selenium dioxide (95.2 g., 0.86 moles) in dioxane (480 ml.) and water (16 ml.) is heated to 50° C.

2,6-Dichloroacetophenone [*Ber, 70,* 921 (1937)] (89.6 g., 0.475 moles) is added over five minutes and the mixture heated at reflux for six hours. After standing at room temperature for 72 hours the mixture is filtered and evaporated *in vacuo*. Two vacuum distillations give the product, 6.0 g. of yellow liquid; b.p. 94–96° C.@0.2 mm.;

$\lambda_{max.}^{KBr}$ 5.85, 5.90 μ;

NMR indicates ca. 10% of starting ketone in the product.

Elemental analysis for $C_8H_4Cl_2O_2$:
Calc'd: C, 47.33; H, 1.99; Cl, 34.93.
Found: C, 47.31; H, 2.38; Cl, 34.62.

EXAMPLE 2

Hydroxy(p-toluoyl)methanesulfonic acid sodium salt

To a solution of (*p*-tolyl)glyoxal, hydrate [Biochem. Z., *279, 459* (1935)] (0.030 moles) in methanol (15 ml.) at room temperature is added all at once a solution of sodium bisulfite (3.15 g., 0.030 moles) in water (25 ml.). After 2 minutes the product begins to precipitate. After cooling at 0° C. for 1 hour the mixture is filtered and the precipitate recrystallized from water/ethanol to give the colorless product; 5.8 g.; m.p. 198–205° C.

$\lambda_{max.}^{KBr}$ 3.01, 5.99μ; $\lambda_{max.}^{EtOH}$ 257 mμ (ϵ 15,000).

NMR has 2.10, 6.22 p.p.m. peaks.

Elemental analysis for $C_9H_9NaO_5S$:
Calc'd: C, 42.88; H, 3.60; S, 12.71; Na, 9.11.
Found: C, 42.80; H, 3.61; S, 12.96; Na, 9.22.

What is claimed is:

1. A process for inhibiting blood platelet aggregation which comprises adding to said blood platelets an effective amount of a compound selected from the group consisting of phenyl glyoxal, *p*-tolyl glyoxal, *p*-methoxyphenyl glyoxal, *p*-chlorophenyl glyoxal, 2,6-dichlorophenyl glyoxal and the hydrates and bisulfite addition salts thereof.

2. The process of claim 1 in which said effective amount is a prophylactic amount incrementally administered at a dosage up to and including 600 milligrams per day, per a 70 kilogram patient.

3. The process of claim 1 in which said compound is administered orally.

4. The process of claim 1 in which said compound is administered intramuscularly.

5. The process of claim 1 in which said compound is phenyl glyoxal, its hyrate or bisulfite addition salts.

6. The process of claim 1 in which said compound is *p*-tolyl glyoxal, its hydrate or bisulfite addition salts.

7. The process of claim 1 in which said compound is *p*-methoxyphenyl glyoxal, its hydrate or bisulfite addition salts.

8. The process of claim 1 in which said compound is *p*-chlorophenyl glyoxal, its hydrate or bisulfite addition salts.

9. The process of claim 1 in which said compound is 2,6-dichlorophenyl glyoxal, its hydrates or bisulfite addition salts.

References Cited

Chem. Abst., 8th collective (1967–1971), vol. 66–75, pp. 5112s and 5113s.

SAM ROSEN, Primary Examiner

U.S. Cl. X.R.
424—315, 331, 333