United States Patent Office

3,189,598

Patented June 15, 1965

3,189,598

FATTY ACID ESTERS OF RIBOFLAVIN

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No Drawing. Filed Apr. 10, 1962, Ser. No. 186,327
Claims priority, application Japan, Jan. 14, 1960, 35/1,097
1 Claim. (Cl. 260—211.3)

The present application is a continuation-in-part of application Serial No. 78,668, filed December 27, 1960, now abandoned.

This invention relates to fatty acid esters of riboflavin and to a process for preparing same.

It has been well known that riboflavin is a slightly water-soluble vitamin and, therefore, has some inconveniences for food-enrichment or pharmaceutical fields.

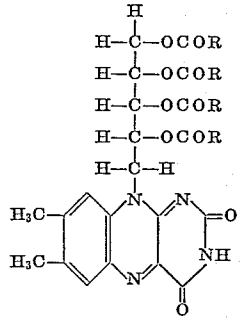
According to this invention I have found that riboflavin may be rendered fat-soluble by esterification with fatty acids. A fatty acid ester of riboflavin can be prepared by reacting riboflavin with an alkanoyl halide having 4 to 20 carbon atoms. The reaction may be carried out in an inert solvent, such as benzene, chloroform or other halogenated hydrocarbons, in the presence of an acid acceptor such as pyridine. Since riboflavin has four free hydroxy groups in the molecule, it is preferable to use more than four moles of the alkanoyl halide per mole of riboflavin. As an alkanoyl halide, I prefer to use those having 4 to 20 carbon atoms such as butyryl, hexanoyl, octanoyl, capryl, lauroyl, myristoyl, palmitoyl and stearoyl chloride or bromide.

The process of the present invention also includes the use of corresponding acid anhydride instead of said acid halide. After the reactants have been heated for the desired length of time, the riboflavin ester is recovered by evaporation of the solvent under reduced pressure or by pouring the reaction mixture into inert solvents in which the riboflavin ester is insoluble.

Among the fatty acid esters, those having lower alkanoyl moiety can be conveniently prepared by directly reacting riboflavin with the alkanoic anhydride in the presence of concentrated perchloric acid. For example, the butyric ester of riboflavin can be obtained in high yield by adding a small amount of 60% perchloric acid into a mixture of riboflavin and butyric anhydride with stirring at room temperature and then pouring the reaction mixture into water.

The fatty acid esters of riboflavin thus prepared may be recovered and purified by recrystallization from ether, benzene or ethanol. The riboflavin esters of this invention are substantially tasteless and odorless, and are quite soluble in many conventional solvents, such as ethanol, pyridine, benzene, chloroform, or neutral fats, in each of which riboflavin itself is almost insoluble.

The riboflavin esters of this invention have the following structural formula:



wherein RCO stands for a straight chain alkanoyl group containing from 4 to 20 carbon atoms.

It has been found that the compounds of this invention possess vitamin B_2 activity. When administered orally, the compounds are easily hydrolyzed with human digestive juices into riboflavin and corresponding fatty acids, and effect vitamin B_2 activity. It has been found that the compounds are hydrolyzed with neither saliva nor gastric juices, but with duodenal juice. Among many of the compounds riboflavin tetrabutyrate is most easily hydrolyzed. This can be illustrated by the test described below.

Each of the substrates was suspended in a mixture of 0.3 ml. of 0.1 M phosphoric acid-buffered solution (pH 8.5) and 0.2 ml. of human duodenal juice at a concentration of 1×10^{-3} M. The substrate solutions were incubated for 30 minutes at 37° C. and the degradation rate of each of the substrates was assayed by the lumiflavin fluorescence method (Yagi, J. Biochem., vol. 43, page 635, 1956). The results are as follows.

Substrate:	Degradation rate (percent)
Riboflavin tetrapalmitate	1.4
Riboflavin tetracaprate	3.5
Riboflavin tetrabutyrate	42.0
Riboflavin tetrapropionate	18.4
Riboflavin tetraacetate	9.4

It has also been found that the compounds of the invention show vitamin B_2 activity on dietary scurvy using male albino rats. Among the compounds, riboflavin tetrabutyrate increases the body weight of rats as much as free riboflavin and it prevents ariboflavinosis remarkably. However, others were found to have little growth-promoting activity.

It has also been found that when the compounds of this invention are administered to the living body by intramusclar injection, the blood level of total riboflavin rises more slowly, and remains at an effective level for a longer period than with free riboflavin. Furthermore, the administered riboflavin is substantially excreted in urine for a longer period of time than is free riboflavin. For example, when 5 mg. of free riboflavin is administered to a rabbit, weighting about 3 kg., by intramuscular injection, the blood level of total riboflavin reaches a peak within 30 to 60 minutes and a substantial quantity of the given riboflavin is excreted in urine within a few hours. However, in the case of riboflavin tetrabutyrate, the blood level of total riboflavin reaches a peak on more than 12 hours after the injection, and 30 to 40% of the given riboflavin is excreted in urine within 24 hours.

From the foregoing, it is apparent that the compounds of this invention are useful in nutritional and medical fields, and sometimes more advantageously than free riboflavin. The invention is further illustrated by the following examples.

Example 1

1 g. of riboflavin was suspended in 300 ml. of a 1:1 mixture of pyridine-chloroform. Into this suspension, 15 g. of palmitoyl chloride in 50 ml. of chloroform was dripped at 0° C. with vigorous stirring within about 1 hour. The mixture was stirred for an additional 14 hours at 33° C. The reaction mixture was concentrated to dryness under reduced pressure and the residue was treated with 100 ml. of 90% pyridine at 70° C. for 15 minutes in order to decompose the remaining palmitoyl chloride into palmitic acid. After the solution was concentrated to dryness, the residue was washed with 200 ml. of methanol to remove palmitic acid and then dissolved in a minimum quantity of absolute ethanol.

The solution was allowed to stand overnight in a refrigerator, whereby riboflavin tetrapalmitate crystallized out. Yield, 2 g., yellow crystals melting at 78.5° C. after recrystallization from absolute ethanol.

Analysis.—Calculated for $C_{81}H_{140}O_{10}N_4$: C, 73.15; H, 10.61; N, 4.21. Found: C, 72.93; H, 10.73; N, 4.41.

Example 2

1 g. of riboflavin was suspended in 300 ml. of 1:1 mixture of pyridine-chloroform. Into this suspension, 10 g. of capryl chloride in 50 ml. of chloroform was dripped at 0° C. with vigorous stirring within about 1 hour. The mixture was stirred for an additional 14 hours at 33° C. The reaction mixture was concentrated to dryness under reduced pressure and the residue was treated with 100 ml. of 90% pyridine at 70° C. for 20 minutes in order to decompose the remaining capryl chloride into capric acid. After the solution was concentrated to dryness, the residue was dissolved in a small amount of ether. The solution was poured into a column of Florisil (an activated mangesium silicate). The column was washed with a small amount of ether and ethanol successively, and then eluted with pyridine. The eluate was condensed to remove pyridine whereby riboflavin tetracaprate was obtained. Yield, 1.5 g. yellow oil.

Example 3

1 g. of riboflavin was suspended in 300 ml. of 1:1 mixture of pyridine-chloroform. Into this suspension, 5.6 g. of butyryl chloride in 50 ml. of chloroform was dripped at 0° C. with vigorous stirring within about 1 hour. The mixture was stirred for an additional 14 hours at 38° C. The reaction mixture was concentrated to dryness under reduced pressure and the residue was treated with 100 ml. of 90% pyridine at 70° C. for 20 minutes in order to decompose the remaining butyryl chloride into butyric acid. After the solution was concentrated to dryness, a small amount of saturated solution of sodium bicarbonate in water was added to the residue and the mixture was extracted with

ether. The extract was dried with sodium sulfate and then ether was removed. The residue was dissolved in ether and insoluble matter was filtered off. The filtrate was condensed to dryness and the residue was treated with petroleum ether on a water-bath, and then filtered. The filter cake was dissolved in ether and a major part of the ether was removed by evaporation. The residue was cooled, whereby riboflavin tetrabutyrate crystallized out. Yield, 1 g., yellowish orange crystals melting at 145° to 147° C. after recrystallization from ether.

Example 4

1 g. of riboflavin was added to 10 ml. of butyric anhydride. The mixture was heated on an oil bath at 130° C. to 140° C. for 6 hours. The resultant solution was concentrated under reduced pressure. Riboflavin tetrabutyrate was crystallized from the residue using ether as a solvent. Yield, 1 g., yellowish crystals melting at 145° C. to 147° C.

Example 5

1 g. of riboflavin was added to 10 ml. of butyric anhydride. To the mixture was added 0.5 ml. of 60% aqueous solution of perchloric acid at room temperature with vigorous stirring. The reaction mixture was poured into 50 ml. of water. After the orange yellowish layer was concentrated under reduced pressure, riboflavin tetrabutyrate was crystallized from ether. 1.5 g. of riboflavin tetrabutyrate melting at 145° C. to 147° C. was obtained.

I claim:

Riboflavin 2',3',4',5'-tetrabutyrate.

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