

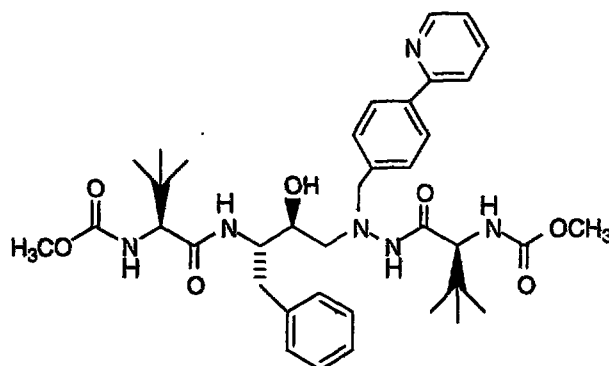
BISULFATE SALT OF HIV PROTEASE INHIBITOR**BACKGROUND OF THE INVENTION**

5

1. Field of the Invention

The present invention provides the novel crystalline bisulfate salt of the azapeptide HIV protease inhibitor of the formula

10

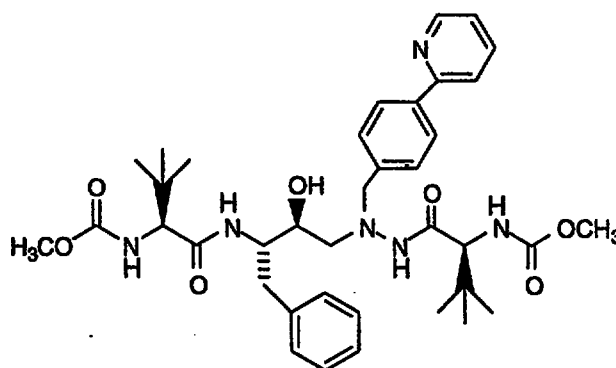
**I**

15 which exhibits unexpectedly superior aqueous solubility/dissolution behavior compared to other salts, and significantly improved oral bioavailability in animals compared to the free base. The bisulfate salt is thus useful for pharmaceutical dosage forms of the above-indicated protease inhibitor, particularly oral dosage forms.

20

2. Background Art

25 Published PCT patent application WO 97/40029 discloses a series of azapeptide HIV protease inhibitors reported to have a high degree of inhibitory activity against the HIV virus. One of the agents included within the scope of WO 97/40029 is the compound having the structural formula



I

5 and the chemical name [3S-(3R*, 8'R*, 9'R*, 12R*)]-3,12-bis(1,1-dimethylethyl)- 8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[[4-(2-pyridinyl)-phenylmethyl]-2,5,6,10,13-pentaazatetradecanedioic acid, dimethyl ester and is under evaluation as a possible second generation HIV protease inhibitor.

10

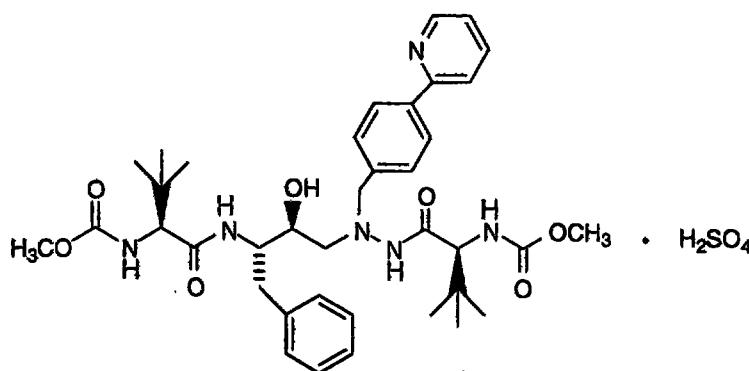
WO 97/40029 discloses the free base form of azapeptide derivatives such as compound I and also various pharmaceutically acceptable acid addition salts. While several organic and inorganic acids are mentioned as possible salt-forming agents, including sulfuric acid, there is no
15 mention of the particular bisulfate salt which is the subject of the present application.

SUMMARY OF THE INVENTION

20

The present invention provides the bisulfate salt of compound I above having the structural formula

25



II

5

DETAILED DESCRIPTION OF THE INVENTION

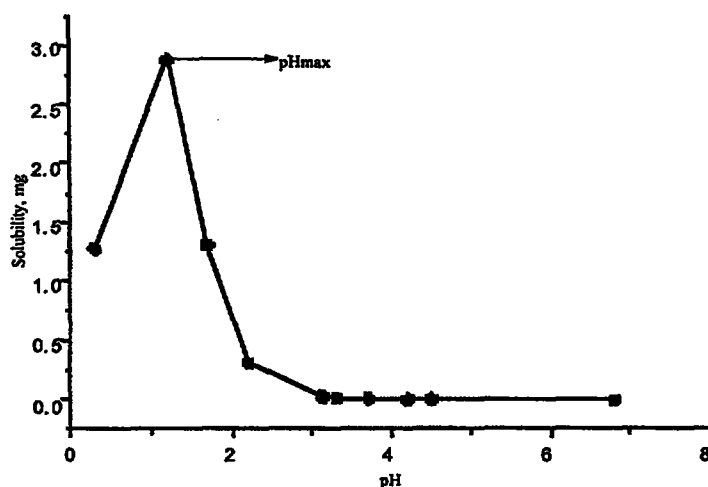
Compound I as disclosed above is a weak organic base with an aqueous solubility of less than 1 $\mu\text{g/mL}$ at $24 \pm 3^\circ \text{C}$. The crystalline free base form as a suspension in water or oil has poor oral bioavailability in animals, probably because of its extremely low solubility in these vehicles.

For development of pharmaceutical formulations, particularly oral dosage forms, the active ingredient must have sufficient oral bioavailability. Since the free base form of compound I did not possess such bioavailability, acid addition salts were explored by the present inventors. A number of commonly used acid addition salts such as the hydrochloride, benzenesulfonate, methanesulfonate, p-toluenesulfonate, phosphate, nitrate, 1,2-ethanedisulfonate, isethionate and sulfate were evaluated, in addition to the bisulfate salt of the present invention. All of these salts in their crystalline form exhibited lower aqueous solubility (1-3 mg/mL or less at $24 \pm 3^\circ \text{C}$) than the bisulfate which had a solubility under the same conditions of approximately 4-5 mg/mL .

Solid state transformation was observed when the other acid addition salts mentioned above were suspended in water, probably due to their dissociation to form the free base. In the majority of cases, this transformation was accompanied by gel formation. Unlike the other salts mentioned above, the extra proton of the bisulfate salt prevents the conversion to the free base which, as mentioned above, is very insoluble in water and has poor oral bioavailability. The unusual solubility

behavior of the bisulfate salt in water is further elaborated in the following.

In general, conversion of salts to the unionized form or vice versa can be explained on the basis of pH-solubility theory. The solubility of the free base in water was determined as a function of pH at $24 \pm 3^\circ \text{C}$ and is shown below. The pH at which the compound exhibits the highest solubility is referred to as pH_{max} and was found to be approximately 1.2. It has been reported in the literature that at $\text{pH} > \text{pH}_{\text{max}}$ of a weakly basic organic compound, the equilibrium solid phase in an aqueous suspension of the compound is the free base. At $\text{pH} < \text{pH}_{\text{max}}$ the equilibrium solid converts to the corresponding salt form. The term "equilibrium solid phase" refers to the undissolved or excess solid in a suspension of the compound in water after sufficient equilibration time. When a salt of a weak base is equilibrated in water in an amount exceeding its solubility limit (i.e., a suspension of the salt in water), the resulting pH of the suspension may fall on either side of the pH_{max} depending on the strength of the acid among other factors. When the resulting pH is greater than the pH_{max} , the suspended solid converts to the free base.



Studies conducted with methane sulfonate and hydrochloride salts, in particular, of the free base confirmed the above described general findings reported in the literature. Amounts in excess of the solubility of these salts were equilibrated in water at $24 \pm 3^\circ \text{C}$ for at least 24 hours. The pH of the suspensions after equilibration was 2.1 ± 0.1 which is greater than the pH_{max} . The undissolved solids from these suspensions were isolated, air-dried, and characterized. By thermal and elemental analysis the undissolved solids from these suspensions were identified as the free base. This behavior was expected based on the pH-solubility profile shown in the graph above and the studies reported in the literature.

When an excess amount of the bisulfate salt was equilibrated in water a modification occurred in the solid phase in equilibrium with solution. However, the undissolved solid phase after equilibration was not the free base, although the pH (1.9 ± 0.2) of the suspension was greater than the pH_{max} and comparable to the pH of the suspensions of methane sulfonate and hydrochloride salts described above. The solid phase after at least 24 hours of equilibration was identified by elemental analysis as a hydrated form of 2:1 salt of the free base form and sulfuric acid (referred to as the sulfate salt). This behavior of the bisulfate salt is unexpected based on pH-solubility theory.

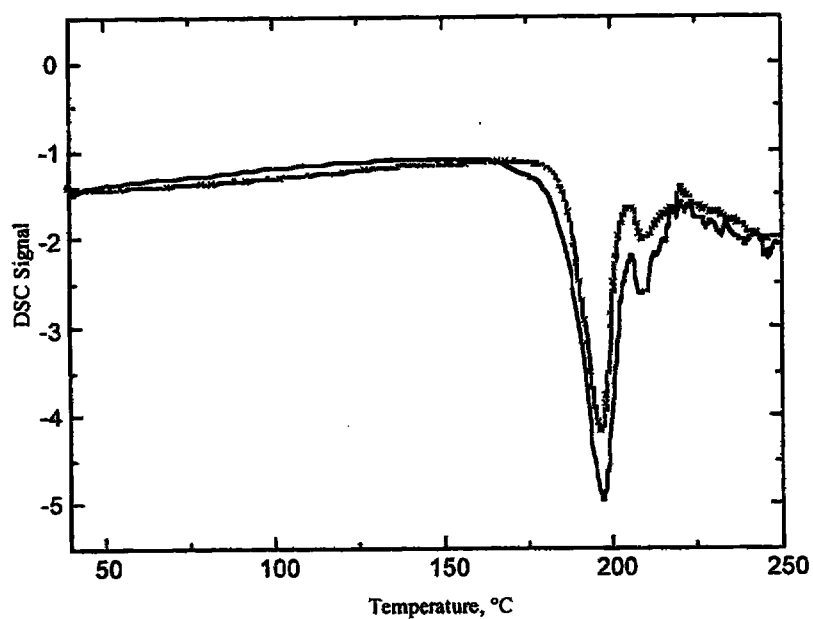
When an excess amount of the sulfate salt, in turn, was equilibrated in water a modification occurred in the solid phase in equilibrium with solution. The undissolved solid from this suspension was isolated, air-dried, and characterized. Thermal and elemental analysis of this undissolved solid phase was similar to that of the free base although the conversion of the sulfate salt to the free base was not as definitive as that of the methane sulfonate and hydrochloride salts. From a pharmaceutical point of view the propensity of salts to convert to the free base in an aqueous environment is not desirable due to the low oral bioavailability of the free base. Thus, the bisulfate salt due its unique solubility behavior in water offered unexpected superiority.

The solubility behavior of the bisulfate salt in water was also unexpected considering the interaction of compound I free base and sulfuric acid in water. For example, the free base exhibited a solubility of less than 1 mg/mL in water at a pH of ~ 1.8 adjusted with sulfuric acid,

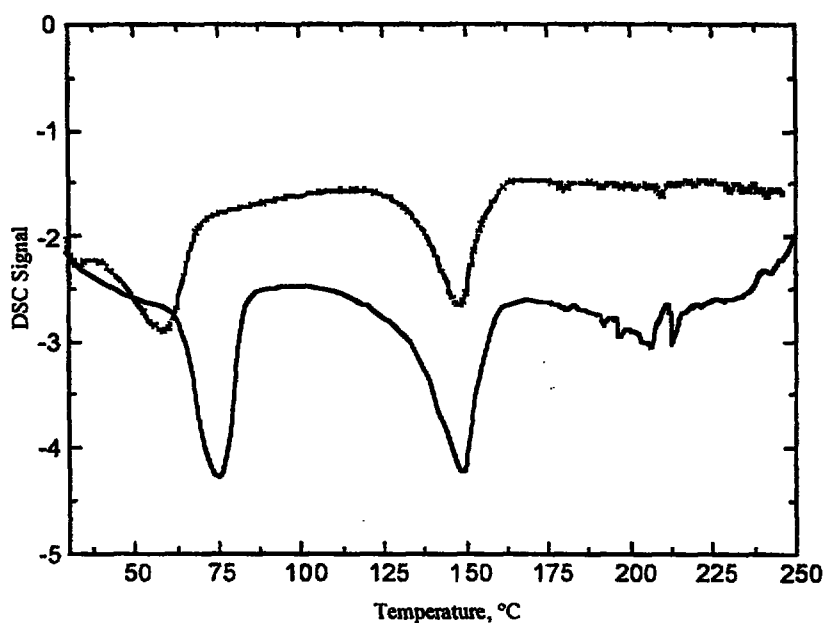
compared to 4-5 mg/mL solubility of the bisulfate salt in water at comparable pH conditions. Based on pH-solubility theory the free base and the salt are expected to exhibit similar solubility at a given pH.

- 5 The enhanced solubility/dissolution behavior of the bisulfate contributes to its improved oral bioavailability in animals relative to the free base. The absolute oral bioavailability of the bisulfate salt was found to be approximately 20% in dogs when administered in unformulated solid form placed in a gelatin capsule. In comparison, the crystalline free
10 base had minimal oral bioavailability in dogs.

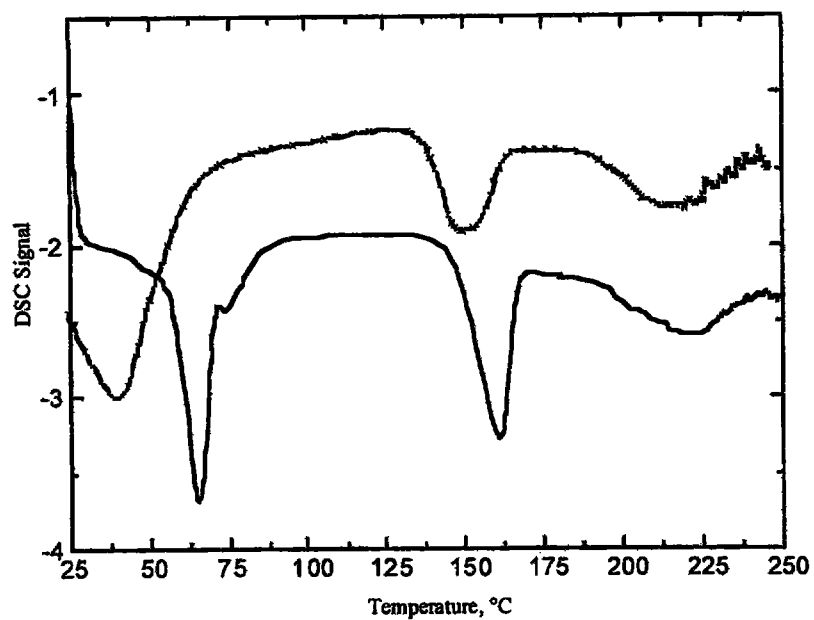
- In addition to optimal solubility, satisfactory physical stability in the solid-state is another desirable property of pharmaceutical salt forms. The term physical stability indicates the ability of the salt form to retain its
15 crystal structure (including solvents of crystallization, if any) under storage/stress conditions. Significant changes in the physical nature of the salt form as indicated by thermal methods such as differential scanning calorimetry are undesirable. The bisulfate salt exhibited excellent solid-state physical stability when stored at 40°C/75% relative humidity (RH) for
20 as long as 9 months as shown in IIa below. Differential scanning calorimetry revealed no significant changes in the thermal behavior of the stressed sample of the bisulfate salt compared to that of the unstressed sample (stored at 2-8°C in a closed container). The methane sulfonate, hydrochloride, and the sulfate salts, on the other hand, showed significant
25 changes in their thermal behavior when stored at 40°C/75%RH for as little as two weeks as shown in II b, c, and d. While differences in physical stability of salt forms is not unusual, the propensity of a particular salt to form solvates (or crystal modifications) and its ability to retain the solvent of crystallization (the physical stability of crystal modifications) under
30 storage/stress conditions cannot be predicted apriori.



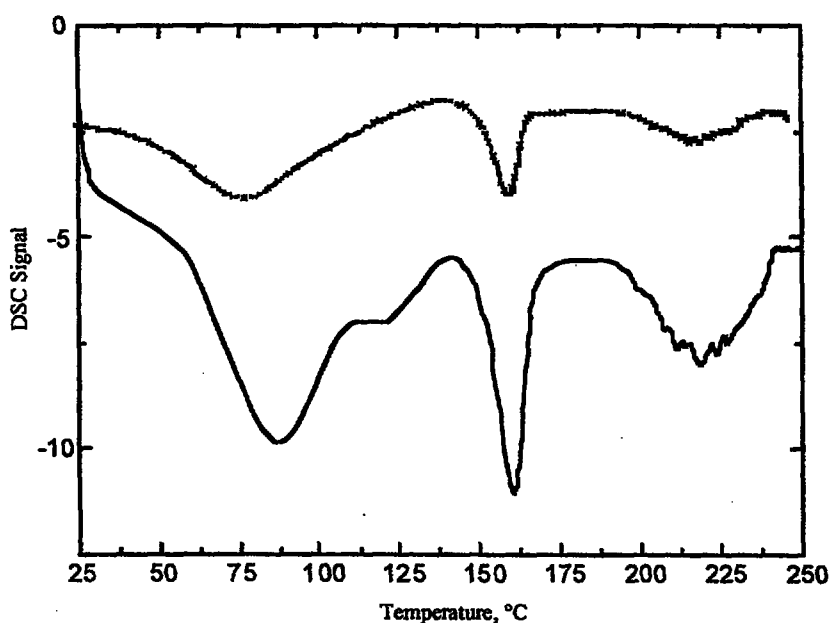
- 5 IIa. Physical stability of the bisulfate salt. The solid line represents the unstressed material. The dotted line represents the material stressed at 40°C/75% RH for 9 months.



IIb. Physical Stability of the hydrochloride salt. The solid line represents the unstressed material. The dotted line represents the material stressed at 40°C/75% RH for two weeks.



IIc. Physical stability of the methane sulfonate salt. The solid line represents the unstressed material. The dotted line represents the material
5 stressed at 40°C/75% RH for two weeks



IId. Physical stability of the sulfate salt. The solid line represents the
5 unstressed material. The dotted line represents the material stressed at
40°C/75% RH for two weeks.

The bisulfate salt may be prepared by forming a solution of free base
10 of compound I with sulfuric acid in solvents such as acetonitrile,
isopropanol, ethanol, or acetone and then isolating the so-produced
bisulfate salt.

Because of its high bioavailability as well as its good crystallinity
15 and stability, the bisulfate salt is very useful in preparing oral dosage
forms of compound I. The examples which follow illustrate preparation
of representative oral formulations.

The bisulfate salt, and formulations thereof, are used as described in
20 WO 97/40029 for the treatment of diseases caused by viruses, especially
retroviruses such as the HIV virus.

DESCRIPTION OF SPECIFIC EMBODIMENTS

Example 1

5 Preparation of Bisulfate Salt From Ethanol

To a 500 mL three-necked round bottomed flask equipped with an overhead stirrer and dropping funnel, 15.013 g (0.0213 mole) of free base compound I and 113 mL of 200 proof ethanol were added with stirring. To
10 this suspension, 1.28 mL concentrated sulfuric acid was added dropwise over 90 seconds. After the addition of sulfuric acid, a clear amber-colored solution was obtained. The solution was polish filtered using #1 Whatman filter paper and washed with 5 mL of 200 proof ethanol. To
15 this solution was added 58 mL of heptane and 37.5 mg (0.25 wt %) of seed crystals of the compound of formula II followed by 55 mL of additional heptane. The resulting mixture was stirred for 6 hours at 300 rpm. The resulting crystal slurry was filtered and washed with 50 mL ethanol/heptane (1:1) solution and dried under vacuum at 60° C
20 overnight to afford 15.11 g of the desired crystalline bisulfate salt (88.4 mole % yield) having formula II above.

Characterizing Properties of Bisulfate Salt

25 Anal. Calcd. for $C_{38}H_{52}N_6O \cdot 1.0 H_2SO_4$: C, 56.84; H, 6.78; N, 10.37; S, 3.99.
Found: C, 56.72; H, 6.65; N, 10.41; S, 3.83.
m.p. 195.0°
H₂O = 0.28% (KF)

30

35

Example 2

Preparation of Bisulfate Salt From Acetone

5 5M H₂SO₄ (8.52 mL, 42.6 mM) was added dropwise to a suspension
of the free base compound of formula I (30.0 g., 42.6 mM) in acetone (213
mL) stirred mechanically in a 50°C oil-bath. A clear solution was obtained
almost immediately. The solution was seeded with crystals of the free
base compound of formula II. After two minutes, a precipitate formed
10 which became a paste. The mixture was stirred at 50°C for one hour, at
25°C for 30 minutes and at 0°C for 2 hours. The solid was filtered and the
first filtrate was used to transfer the remaining material in the flask to the
filtration funnel. The product was washed with acetone, then heptane,
and dried under vacuum overnight to give 31.48 g (corrected yield 92%) of
15 the bisulfate salt of formula II, m.p. 198-199°C dec.

Anal. Calcd. C₃₈H₅₂N₆O₇•1.0 H₂SO₄•0.2 H₂O : C, 56.59; H, 6.80; N, 10.42; S,
3.98; H₂O, 0.45.

Found: C, 56.66; H, 6.78; N, 10.50; S, 4.20; H₂O, 0.45 (KF).

20

Example 3

Preparation of Bisulfate Salt From Isopropanol

25

Aqueous sulfuric acid (5.0 M, 0.20 mL, 1 mM) was added to a
suspension of the free base compound of formula I (0.704 g, 1.00 mM) in
isopropanol (4.0 mL) chilled in an ice-bath. The ice-bath was removed
and the mixture stirred at room temperature. The suspension had
30 dissolved after 15 minutes. The solution was seeded with crystals
prepared as in Examples 1 or 2 above and stirred for 5 hours. The solid
was filtered and the filtrate was used to transfer the solid from the flask to
the funnel. The product was washed with heptane and dried under
vacuum to give 0.752 g of crystalline bisulfate salt of formula II, yield 90%,
35 m.p. 160-190°C, dec.

Anal. Calcd. for $C_{38}H_{52}N_6O_7 \cdot 1.0 H_2SO_4 \cdot 2.0 H_2O$; C, 54.40; H, 6.97; N, 10.02; S, 3.82; H_2O , 4.29.

Found: C, 54.25; H, 6.73; N, 10.02; S, 3.67; H_2O , 4.53 (KF).

- 5 The crystals obtained from isopropanol showed a powder x-ray diffraction pattern different from the crystals obtained from acetonitrile, ethanol-heptane or acetone. They are now referred to as Type-II crystals. The Type-I crystals appear to be an anhydrous/desolvated crystalline material while the Type-II crystals are a hydrated, hygroscopic crystalline
- 10 form.

Example 4

15 Preparation of Capsule Formulations of Bisulfate Salt

A. Capsules (50 and 200 mg free base equivalent)

- 20 Capsules are provided for oral administration in which the capsule is a size #0, gray, opaque, hard gelatin capsule containing the bisulfate salt of formula II formulated as a wet granulation with lactose, crospovidone and magnesium stearate.

25 B. Capsules (100 mg free base equivalent)

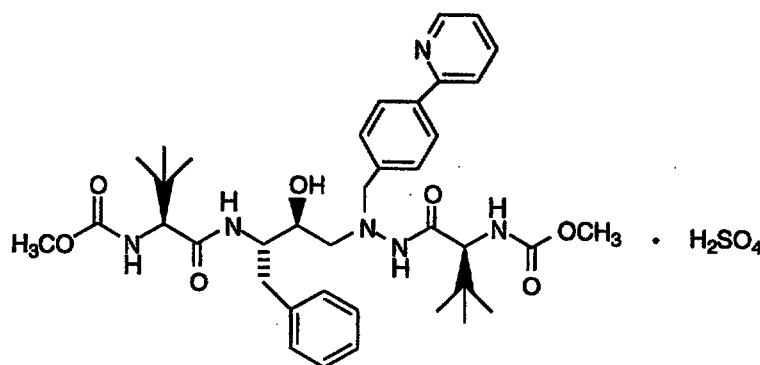
- 30 Capsules are provided for oral administration in which the capsule is a size #0, gray, opaque, hard gelatin capsule containing the bisulfate salt of formula II suspended in Gelucire 44/14. Gelucire 44/14 is a saturated polyglycolized glyceride consisting of mono-, di- and triglycerides and mono- and di-fatty acid esters of polyethylene glycol. Capsules are prepared by melting Gelucire 44/14 at 45-70° C followed by addition of the bisulfate salt with stirring. The molten mixture is filled into hard gelatin capsules and allowed to cool and solidify.

CLAIMS

We claim:



- 5 1. The bisulfate salt having the formula



II

10

2. A pharmaceutical dosage form comprising the bisulfate salt of
Claim 1 and a pharmaceutically acceptable carrier.

15