Table II—Comparison of Analytical Data[a] (Percent Purity)

Sample	GLC Procedure	Colorimetric Assay[b]	Iodometric Assay[c]
Compound I			
1	98.7	100.3	98.5
2	99.3	99.5	98.6
3	95.2	96.7	97.5
Compound II			
4	95.2	94.6	90.0
5	88.2	87.7	88.7
6	96.6	93.6	93.3
7	99.7	97.4	94.6
8	57.8[d]	81.5	86.5
9	93.3	93.4	89.3

[a] Standard deviations of known procedures are calculated on our results (average of at least two determinations). [b] For I samples, $\sigma = \pm 1.30\%$. For II samples, $\sigma = \pm 0.96\%$. [c] For I samples, $\sigma = \pm 1.25\%$. For II samples, $\sigma = \pm 3.04\%$. [d] The sample has a content of 29.3% of IV. The ratio of II to IV was 2.08 as determined by NMR spectroscopy *versus* 1.97 as calculated by GLC.

strong silylating agents such as *N,O*-bis(trimethylsilyl)formamide (about 30% of IV), *N*-trimethylsilylimidazole (60% of IV), and *N*-trimethylsilylacetamide (about 10% of IV). In the last two trials, incomplete silylation was obtained.

Hexamethyldisilazane alone or with trimethylchlorosilane[9] does not form IV during derivatization of II (12), which is, however, incomplete. Quantitative silylation on the amino group, which is much more difficult (14) to react than the carboxylic group, was attained in a second step by adding V at room temperature. This procedure leaves the double bond of the dihydrothiazinyl ring unaltered. Furthermore, a shorter column was finally chosen to minimize previously observed decomposition phenomena. The analytical data are reported in Table I.

The analytical results of samples of I and II obtained by GLC (Figs. 1 and 2), compared with the colorimetric determination of Marrelli (9) and with the iodometric assay of Alicino (8), are reported in Table II. The GLC determinations of these substrates are independent of related substances such as IV, which interfere in the chemical methods (see Sample 8, Table II). Furthermore, IV as an impurity can be detected accurately up to 0.5% in samples of II by this GLC method.

The good agreement between the data reported in Table II supports the use of the GLC procedure and indicates that it might supplement the chemical methods.

[9] The 1:3 molar ratio of these two silylating reagents was found to be the most suitable after preliminary experiments.

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Fluorescence Properties of Glafenine

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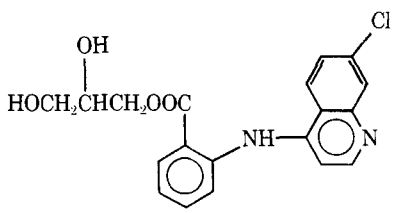
Abstract □ The fluorescence properties of glafenine are partly caused by the anthranilic acid nucleus of the molecule. Correlations are made between fluorescence capacities and UV absorbance. Analytical determinations can be carried out in different solvents, *e.g.*, ether, benzene, and ethanol. Linearity of the emission intensity with the concentration, limiting detectable sample concentrations, and Stokes shifts are reported.

Keyphrases □ Glafenine—fluorescence characteristics in various solvents, effect of pH □ Fluorescence characteristics—glafenine in various solvents, effect of pH □ Analgesics—glafenine, fluorescence characteristics in various solvents, effect of pH

Glafenine[1] (I), 2-[(7-chloro-4-quinolinyl)amino]benzoic acid 2,3-dihydroxypropyl ester, is used frequently for its analgesic properties. It is a pale-yellow powder, mp 165°, and is nearly insoluble in water, slightly soluble in organic solvents, and soluble in aqueous acid solutions. Since this substance contains the anthranilic acid nucleus, the following report is concerned with the question of whether this molecule possesses native fluorescence characteristics.

Anthranilic acid, *o*-aminobenzoic acid, exhibits a strong native fluorescence (λ_{exc} = 300 nm and λ_{em} = 405 nm at pH 7) (1), for which pH dependence (2), decay time (3), and assay in human urine (4) have been reported.

Solutions of glafenine in diluted mineral acids show an intense yellow color but do not fluoresce. In neutral and



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[1] Glifanan, 200-mg tablets, Roussel-Labunis, France.

Table I—Fluorescence Characteristics of Glafenine in Various Solvents (5 μg/ml)

Solvent	λ_{exc}, nm	λ_{em}, nm	Intensity[a]	Stokes Shift, cm^{-1} (5, 6)
Benzene	330	392	20	4790
Ether	250[b], 327	400	17	5580
Chloroform	250[b], 340	436	10	6480
2-Propanol–10% (v/v) buffer, pH 9[c] (0.1 *N* glycine–sodium hydroxide)	340	410	6	5020
2-Propanol–10% (v/v) buffer, pH 4 (0.1 *N* citrate–hydrochloric acid)	265[b], 355	450	2	5950
2-Propanol–10% (v/v) buffer, pH 1 (0.2 *N* potassium chloride–hydrochloric acid)	300	407	0.5	8760
Ethanol	245[b], 336	439	6	6980
Methanol	245[b], 336	425	2	6230
Methanol–10% (v/v) ammonia[c] (28% w/w)	273[b], 365	455	0.5	5420
Acetone	330	430	0.5	7050
Sulfuric acid (10–0.005 *N*)	—	—	No signal	—

[a] Referred to a solution of quinine sulfate in a concentration of 1 μg/ml in 0.5 *N* sulfuric acid, of which the relative fluorescence intensity is 100, measured simultaneously. [b] Secondary excitation value with much less intensity. [c] Care should be taken for possible precipitation in the alkaline medium.

alkaline media, however, a remarkable blue fluorescence is observed on irradiation at 350 nm.

DISCUSSION

Since the fluorescence phenomenon basically is preceded by the light absorption of the molecule, the visible and UV spectra of this compound were checked in various solvents to correlate them with the activation spectra. The following absorption maxima can be assigned: ethanol, 355 (ε 19,600) and 255 (ε 17,000) nm; acetone, 355 (ε 18,700) and 255 (ε 5600) nm; chloroform, 360 (ε 22,900) and 255 (ε 18,100) nm; ether, 351 (ε 19,300) and 255 (ε 19,000) nm; and benzene, 360 (ε 14,900) and 255 (ε 4100) nm.

Activation and fluorescence spectra were taken in various solvents at different pH values. No fluorescence emission occurred in the lower pH range; from pH 4 to more alkaline media, fluorescence occurred with a maximum at pH 9–10.

Table I illustrates excitation and emission maxima in order of decreasing intensity. The Stokes shift, a measure of the energy dissipated during the lifetime of the excited state before return to the ground state, increased with increasing solvent polarity, and the compound behaved as an organic base (Table I).

When comparing wavelength maxima of light absorption and those corresponding with the activation maxima, it can be noticed that fluorescence emission occurred most intensely after illumination at 320–360 nm, the wavelengths of highest molar absorptivities, considering instrumental artifacts of wavelength calibration. However, the energetically most favorable 245–265-nm wavelengths of absorption distinctly were of inferior value in the fluorescence process. When ε was low (<6000), no secondary excitation maximum could be assigned in the fluorescence process.

Figure 1 illustrates the dual excitation pattern and the emission spectrum of glafenine dissolved in ether. The relation between the relative emission intensity and the analytical concentration is shown in Fig. 2. The signal was linear up to 8 μg/ml, followed by the self-absorption effect, causing a negative curvature from 15 to 20 μg/ml. The limiting detectable sample concentration estimated graphically (7) is 8×10^{-2} μg/ml. Analogous results were obtained from benzene: λ_{exc} = 330 nm, λ_{em} = 392 nm, linearity up to 8 μg/ml, negative curve at 25 μg/ml, and limiting detectable sample concentration 5×10^{-2} μg/ml. For ethanol, the results were: λ_{exc} = 336 nm, λ_{em} = 439 nm, linearity up to 8 μg/ml, negative curve at 15–20 μg/ml, and limiting detectable sample concentration 1×10^{-1} μg/ml.

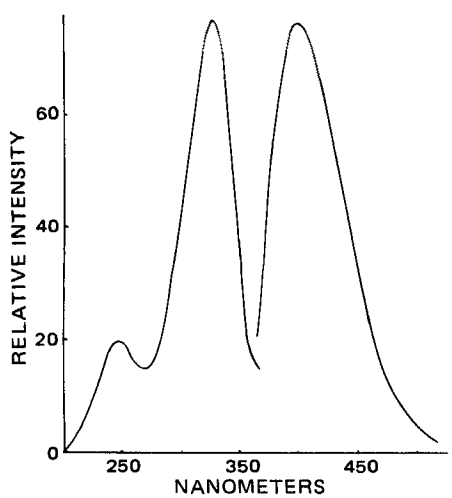

Figure 1—*Excitation and emission spectra of glafenine in ether (10 μg/ml); λ_{exc} = (250, secondary value with much less intensity) 327 nm and λ_{em} = 400 nm.*

The absence of any clear fluorescence signal of the glafenine molecule in the aqueous acid medium illustrates that the native fluorescence capacities belong to the undissociated free base, in which the chlorine atom causes a decrease of the fluorescence quantum efficiency.

The native fluorescence of glafenine in ether was used for determining this compound directly in tablets after extraction with ether. Samples were diluted to a concentration of 5 μg/ml and measured at the appropriate excitation and emission wavelengths. The time period of extraction of the tablets depends on the room temperature and on the particle size of the powdered tablets. In the present experiments, magnetic stirring periods for extraction varied between 1 and 2 hr.

A relative standard deviation of 1.7% was obtained.

EXPERIMENTAL[2]

Apparatus—A recording spectrophotofluorometer was used for determining excitation and emission spectra and for measuring fluorescence intensities. Spectra were not corrected for variations in lamp intensity or photomultiplier sensitivity.

Chemicals—All chemicals[3] were analytical grade.

Preparation of Solutions for Fluorescence Measurement—All

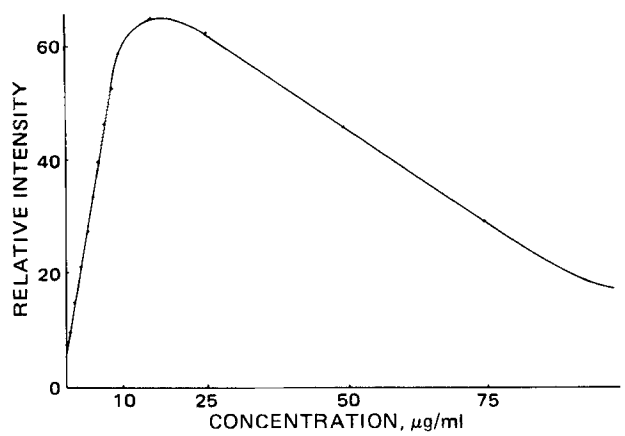

Figure 2—*Analytical curve of glafenine in ether.*

[2] The instruments used were: an Aminco-Bowman spectrofluorometer, Catalog No. 4-8203 DE, American Instrument Co., Silver Spring, Md.; a Hanovia 150-w xenon arc lamp; two grating monochromators; 1 × 1-cm quartz cells; photomultiplier R 446 S with high voltage of 0.7 kv and slits varying between 0.5 and 2 mm; a Zeiss DMR 21 spectrophotometer; and a Camag TL 900 Universal UV lamp at 254 and 350 nm.

[3] E. Merck, Darmstadt, Germany.

solutions measured fluorometrically contained 1×10^{-2}–100 μg of glafenine/ml of solvent. All glassware should be free of fluorescent contaminants and, therefore, frequently rinsed with distilled water; quartz sample cells are preferably cleaned with nitric acid followed by distilled water.

Determination of Glafenine in Tablets—Ten tablets were weighed accurately, and the average weight was calculated. They were brought to a homogeneous fine powder in a mortar, and a quantity equivalent to 25 mg of glafenine was transferred into a 500-ml conical flask. About 450 ml of ether was added, and the mixture was stirred with a magnetic stirrer for 2 hr. After filtration on a paper filter, the filtrate was diluted to 500 ml with ether. Then 10 ml of this solution was diluted to 100 ml with ether.

An analogous standard solution was prepared by dissolving 25 mg of glafenine in 500 ml of ether; 10 ml of this solution was diluted to 100 ml with ether.

It is advisable to extract the tablets simultaneously with the preparation of the standard solution or during at least the same period to avoid incomplete extraction.

Pure ether was used as a blank solution. Fluorometric measurement was performed at 327-nm excitation and 400-nm emission.

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Gonadotropin-Inhibitory Contaminants in Partially Purified Pharmaceutical Preparations of Human Chorionic Gonadotropin

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Abstract □ Various commercial preparations of partially purified human chorionic gonadotropin, inactivated by heating, inhibited the uterine growth induced in immature mice with the same active gonadotropins as well as spontaneous uterine growth. The more purified preparations of chorionic gonadotropin failed to produce these effects after inactivation by boiling, suggesting that the inhibitory activity is not generated from gonadotropin by the procedure but may be related to some contaminant similar to the gonadotropin-inhibitory substance previously found in human urine.

Keyphrases □ Gonadotropin, human chorionic—various commercial preparations, presence of inhibitory contaminants □ Contaminants, inhibitory—presence in various commercial preparations of human chorionic gonadotropin □ Inhibitory contaminants—presence in various commercial preparations of human chorionic gonadotropin

Human chorionic gonadotropin (I) for clinical purposes is a concentrate of the urine of pregnant women containing several hundred to 1000 international units (IU)/mg. It is obtained by a procedure that may also extract the gonadotropin-inhibitory substance (II) reported previously (1–3). In the present work, the occurrence of II was studied in various commercially available brands of I and in highly purified preparations. Preliminary results were reported previously (4).

EXPERIMENTAL

Five preparations of human chorionic gonadotropin were studied: partially purified preparations Ia[1], Ib[2], and Ic[3] and highly purified preparations Id[4] and Ie[5], with an activity of 13,000 and 10,000 IU/mg, respectively. These materials were dissolved in distilled water (500 IU/ml), pH 5–6, and boiled under reflux in a water bath for 1 hr to inactivate the gonadotropin activity (3). The final volume was adjusted to the desired concentration by adding water.

Biological assays were done in immature mice, 7.5–10.0 g, of the Balb-c strain. Different doses of boiled I preparations were injected, alone or with unboiled I, at different sites or mixed in the same syringe. The total dose of boiled and unboiled I was given in five subcutaneous injections, 0.2 ml each, for 3 days. Necropsy was performed on the 4th day, 24 hr after the last injection.

The mice were killed with ether. The uteri were dissected clean of surrounding tissue, dried by blotting on filter paper, and weighed on a precision balance to the nearest hundredth of a milligram. The Student *t* test was used to compare the mean weights of the uteri obtained from the different experimental groups.

RESULTS AND DISCUSSION

The less pure preparations of I, with 1000 IU/mg, inactivated by boiling, inhibited the vaginal opening and uterine growth induced with unboiled I in immature mice (Table I). This effect appeared when both preparations were injected together or separately in different subcutaneous sites. No signs of toxicity were evidenced, and the growth of the treated and untreated mice was similar. The heat-inactivated I also in-

[1] Apoidine, Parke-Davis.
[2] APL, Ayerst Laboratories.
[3] Profasi, Serono, Italy.
[4] Lot E 231 TEBZ, Serono, Italy.
[5] Donated by Professor C. H. Li.