the antibacterial action of both antibiotics is similar. Moreover, the breakdown products comprise only a small (nondetectable) fraction of the tetracyclines after *in vivo* administration (14). In many cases, the fluorometric method, as presented here, may become the method of choice.

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Sepiolite, a Potential Excipient for Drugs Subject to Oxidative Degradation

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Received May 5, 1980, from the *Departments of *Agronomy and ‡Industrial and Physical Pharmacy, Purdue University, West Lafayette, IN 47907.* Accepted for publication July 29, 1980.

Abstract □ Sepiolite, a member of the fibrous mineral group of clays, is relatively free of surface ferric iron and does not accelerate significantly the oxidative degradation of hydrocortisone. The compatibility of sepiolite with drugs that can undergo oxidative degradation is in sharp contrast to the catalytic effect of attapulgite, another fibrous mineral, which contains a significant amount of surface ferric iron and which therefore accelerates oxidative degradation. Sepiolite adsorbs hydrocortisone by a weak adsorption mechanism, which was shown by IR spectroscopy to be chiefly due to hydrogen bonding. However, accelerated oxidative degradation of the adsorbed hydrocortisone does not occur. Maximum adsorption occurs at pH 7–8.5. Desorption occurs readily by washing with water. Sepiolite has similar rheological properties to attapulgite. The results of this study suggest that sepiolite may be useful as a pharmaceutical excipient for drugs that undergo oxidative degradation.

Keyphrases □ Sepiolite—potential as excipient for drugs subject to oxidative degradation □ Excipients—potential of sepiolite as excipient for drugs subject to oxidative degradation □ Clays—sepiolite, potential as excipient for drugs subject to oxidative degradation □ Adsorption—sepiolite, potential excipient evaluated for use with drugs subject to oxidative degradation

A recent study (1) demonstrated that hydrocortisone is adsorbed weakly by attapulgite and subsequently undergoes oxidative degradation, catalyzed by both adsorbed iron oxides and hydroxides as well as by structural ferric iron at the clay surface. Other clays such as montmorillonite (2–4) and hectorite (5, 6) promote the oxidation of organic compounds. Surface-adsorbed contaminants or structural ferric iron at the clay surface have been suggested as being responsible for the oxidation of organic materials by these clays.

BACKGROUND

Attapulgite, which frequently is termed palygorskite in clay mineralogy literature, is a member of the fibrous mineral group of clays. The fibrous minerals are similar to the smectite group of clays, which includes montmorillonite, hectorite, and saponite, since they are 2:1 type minerals consisting of a layer of magnesium octahedra sandwiched between two silica tetrahedral sheets. However, their properties are significantly different from the smectite group since crystal growth is limited to the C-dimension, resulting in ribbons of the 2:1 layer attached at their longitudinal edges. A cross section of the fiber gives a checkerboard arrangement of ribbons and voids with no possibility of expansion. In addition, the fibrous minerals have little or no true cation-exchange capacity. However, because of the very thin nature of the ribbons, the external surface area is moderately high. Fibrous minerals also are very porous due to the channels between the ribbons. However, little adsorption occurs within the pores since the dimensions of the pores can accommodate only small molecules such as water, ammonia, and lower alcohols (7).

Sepiolite also belongs to the fibrous mineral group of clays and has an ideal formula, external surface area, internal surface, and channel dimensions that are very similar to attapulgite (Table I). However, there is a striking difference in the ferric iron content of natural samples. Sepiolite samples contain much less ferric iron than is found in attapulgite samples. Since the surface ferric iron is responsible for catalyzing the oxidative degradation, it was decided to investigate the effect of sepiolite on the oxidative degradation of hydrocortisone. In addition, since sepiolite has not been mentioned as a pharmaceutical excipient or as a GI adsorbent, the rheological properties of sepiolite suspensions were compared to attapulgite suspensions to determine if sepiolite could be considered for such use.

EXPERIMENTAL

Materials—All chemicals were official or reagent grade. The vallecas sepiolite studied was obtained from a deposit near Madrid, Spain, and was used as received. X-ray diffractograms indicated that sepiolite was the major mineral present, although a small amount of calcite also was found. The total iron content was determined by hydrofluoric acid dissolution (8).

Hydrocortisone Assay—A high-pressure liquid chromatographic (HPLC) method that was recommended for the analysis of hydrocortisone tablets (9) was modified slightly for this study. The liquid chro-

Table I—Comparison of Formula and Dimensions of Sepiolite and Attapulgite

	Sepiolite	Attapulgite
Ideal formula	$(OH_2)_4(OH)_4Mg_8Si_{12}O_{30}8H_2O$[a]	$(OH_2)_4(OH)_2Mg_5Si_8O_{20}4H_2O$[b]
Natural sample formula	$(OH_2)_4(OH)_4(Si_{11.92}Al_{0.08})(Al_{0.52}Fe^{3+}_{0.02})(Mg_{6.98}Fe^{2+}_{0.08})$[c]	$(OH_2)_4(OH)_2(Si_{0.83}Al_{0.17})(Al_{1.36}Ti_{0.07}Fe^{3+}_{0.37})(Fe^{2+}_{0.03}Mg_{1.98})$[b]
External surface area, m^2/g	400[d]	280[d]
Internal surface area, m^2/g	500[d]	635[d]
Channel dimensions, Å	3.7×10.6[d]	3.7×6.4[d]

[a] K. Brauner and A. Preisinger, *Tschermaks Mineral Petrogr. Mitt.*, **6**, 120 (1956). [b] W. F. Bradley, *Am. Miner.*, **25**, 405 (1940). [c] J. L. Ahlrichs, C. Serna, and J. M. Serratosa, *Clays Clay Miner.*, **23**, 119 (1975). [d] Reference 7.

matograph[1] was equipped with a UV detector operating at 254 nm and a 20-μl injector loop[2]. A commercially packed octadecylsilane[3] column was used with acetonitrile–water (35:65) as the mobile phase. The operating parameters were: flow rate, 1 ml/min; pressure, 1000–1200 psi; temperature, ambient; and UV attenuator, 0.02 aufs. Linear calibration curves were used to quantify the hydrocortisone content of the aqueous phase, while the relative concentration of the observed degradation products was characterized by peak heights.

Changes in the A-ring of hydrocortisone were monitored by UV spectrometry at 254 nm.

IR Analysis—Self-supporting films were prepared for IR analysis[4] by pipetting appropriate volumes of either sepiolite or hydrocortisone–sepiolite suspensions onto polyethylene terephthalate[5] and air drying at room temperature (10).

Hydrocortisone was prepared for IR analysis as a potassium bromide pellet.

Kinetic Studies—The sepiolite concentration was selected as representative of the range of clay usually used in pharmaceuticals; the hydrocortisone concentration was chosen to be below the solubility limit, 280 μg/ml at 25° (11), to ensure complete solubility during the kinetic studies. Thus, 300 mg of sepiolite was mixed with 25 ml of an aqueous solution of hydrocortisone (200 μg/ml) in a 50-ml stoppered centrifuge tube. The pH was adjusted with hydrochloric acid to pH 8.4 to compare the chemical stability of hydrocortisone in the presence of sepiolite with the results reported previously (1) for hydrocortisone–attapulgite suspensions. The samples were aged in a constant-temperature shaker bath at 23°. At appropriate intervals, aliquots were taken and centrifuged at 6000 rpm, and the supernate was filtered and analyzed by HPLC and UV spectrometry. The pH was 8.4 at each sampling.

Aqueous solutions of hydrocortisone (200 μg/ml) adjusted to pH 8.4 with sodium hydroxide were aged with the hydrocortisone–sepiolite suspensions and served as controls.

Adsorption Isotherm—Hydrocortisone–sepiolite suspensions were prepared as described, except that hydrocortisone concentrations of 1–200 μg/ml were used. The suspensions were equilibrated in the constant-temperature shaker bath at 23° for 24 hr. The amount of hydrocortisone adsorbed was calculated from the change in the hydrocortisone concentration of the supernate after equilibration as determined by HPLC assay and UV spectrometry at 254 nm.

Desorption studies were performed by recovering the equilibrated sepiolite from the adsorption study and resuspending the sepiolite in sufficient water, adjusted to pH 8.4, to achieve the original sepiolite concentration. After equilibration for 30 min in the constant-temperature shaker bath at 23°, the procedure was repeated to give sepiolite samples, which were washed one, two, or three times. The hydrocortisone concentration desorbed in each washing was determined by HPLC.

Effect of pH on Adsorption—Eight hydrocortisone–sepiolite suspensions were prepared at pH 2.4, 3.2, 5.3, 6.8, 7.8, 8.5, 9.2, and 9.8 by adding 20 ml of a hydrocortisone stock solution (250 μg/ml) to 300 mg of sepiolite. Each suspension was adjusted to the desired pH with hydrochloric acid or sodium hydroxide. The suspensions were diluted to 25 ml with water to produce sepiolite suspensions containing 200 μg of hydrocortisone/ml. The suspensions were equilibrated for 24 hr in the constant-temperature shaker bath at 23°. The concentration of hydrocortisone adsorbed was determined as described under *Adsorption Isotherm*.

Viscosity Measurement—The rheological properties of 1 and 2% sepiolite suspensions were compared to 1 and 2% attapulgite suspensions using a rotational viscometer[6] with spindle 1. The suspensions were prepared by sonicating each suspension for 1 min.

RESULTS AND DISCUSSION

Hydrocortisone exhibited very slow degradation in aqueous solution at pH 8.4, which is consistent with previously reported stability results (1, 12, 13). In a sepiolite suspension, the hydrocortisone content of the aqueous phase decreased from 200 to 125 μg/ml during the 1st hr and very slowly during the next 170 hr (Fig. 1). As noted in Fig. 1, identical stability results were obtained by HPLC and UV analysis.

The previously reported (1) degradation profile of hydrocortisone in an attapulgite suspension as determined by HPLC analysis also is shown in Fig. 1. The initial decrease in the hydrocortisone content as a result of interaction with attapulgite was less than was observed in the presence of sepiolite. However, the hydrocortisone content of the aqueous phase of the attapulgite suspension decreased substantially during the entire 170 hr of the study. Furthermore, except for a small initial decrease, UV spectroscopy showed no change in the hydrocortisone concentration when the hydrocortisone–attapulgite suspensions were aged at 23, 38, 50, and 69° (1).

The HPLC and UV analyses of the hydrocortisone–sepiolite suspensions suggested that hydrocortisone is adsorbed by sepiolite, producing

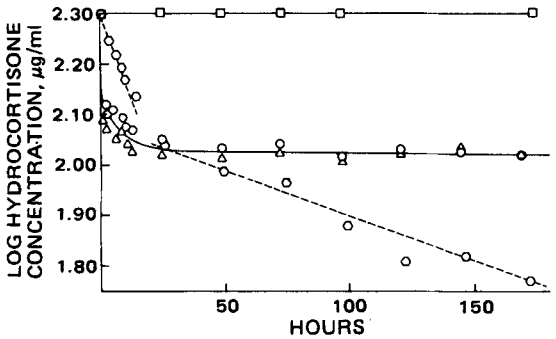

Figure 1—*Change in hydrocortisone concentration of aqueous phase (theory = 200 μg/ml) during aging at pH 8.4 and 23°. Key: □, hydrocortisone solution determined by HPLC; △, sepiolite suspension determined by HPLC; ○, sepiolite suspension determined by UV spectrometry; and ○, attapulgite suspension determined by HPLC from Ref. 1.*

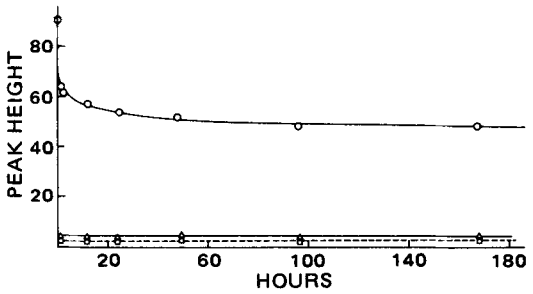

Figure 2—*Relative concentration of hydrocortisone and degradation products in sepiolite suspensions at pH 8.4 during aging at 23°. Key: ○, hydrocortisone; △, degradation product with a retention time of 2.5 min; and □, degradation product with a retention time of 11 min.*

[1] Model ALC 202, Waters Associates, Framingham, Mass.
[2] Rheodyne, Berkeley, Calif.
[3] Partisil-10 ODS, Whatman Inc., Clifton, N.J.
[4] Model 180, Perkin-Elmer Corp., Norwalk, Conn.
[5] Mylar.
[6] Model RVT, Brookfield Engineering Laboratories, Stoughton, Mass.

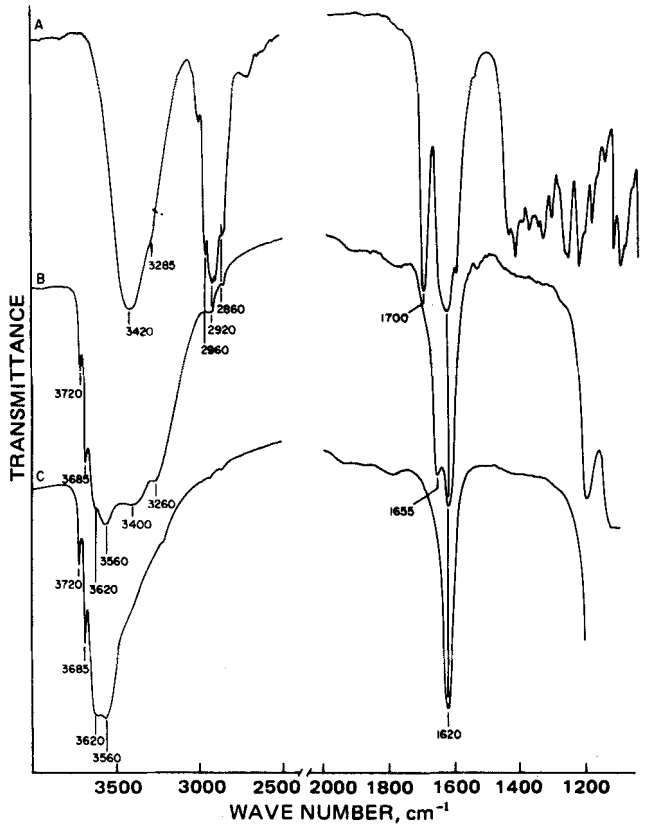

Figure 3—*IR spectra of hydrocortisone in a potassium bromide pellet (A), a film of equilibrated hydrocortisone–sepiolite suspension at pH 8.4 (B), and a film of sepiolite (C).*

an immediate decrease in the hydrocortisone concentration. However, little degradation of hydrocortisone appears to occur in the sepiolite suspension. This hypothesis is confirmed by analysis of the peaks appearing in the high-pressure liquid chromatogram. With the HPLC system used, hydrocortisone has a retention time of 8 min, while the two initial degradation products of hydrocortisone have retention times of 2.5 and 11 min (1). The peak height of the hydrocortisone peak dropped initially for the sepiolite suspension but changed relatively little on aging (Fig. 2). However, the peak height for the peaks corresponding to the two initial degradation products was very low and increased only slightly on aging. Thus, in contrast to the hydrocortisone–attapulgite suspension (1), it does not appear that hydrocortisone degrades very rapidly in the presence of sepiolite. The initial decrease in the hydrocortisone concentration seems to be due to adsorption of hydrocortisone by sepiolite rather than to accelerated degradation of hydrocortisone.

The adsorption of hydrocortisone by sepiolite was examined by IR spectroscopy. Figure 3A shows the IR spectrum of hydrocortisone in a potassium bromide pellet. The IR spectrum of sepiolite is shown in Fig. 3C. The IR spectrum of the hydrocortisone–sepiolite suspension at pH 8.4 (Fig. 3B) shows the hydroxyl stretching vibration of sepiolite at 3720 and 3685 cm^{-1}. Water displayed asymmetric hydroxyl stretching bands at 3620 and 3560 cm^{-1} and a hydroxyl bending vibration at 1620 cm^{-1}. Hydrocortisone absorption bands were evident at 3400, 3260, 2960, 2920, 2860, and 1655 cm^{-1}. The IR spectrum of hydrocortisone–sepiolite

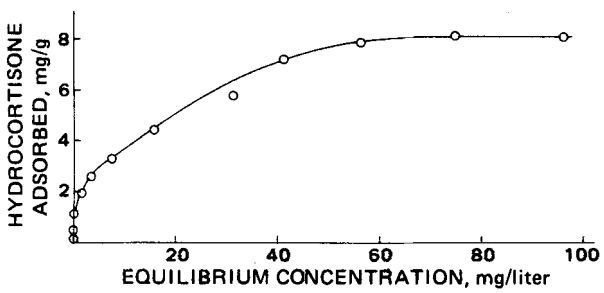

Figure 4—*Adsorption isotherm for hydrocortisone by sepiolite.*

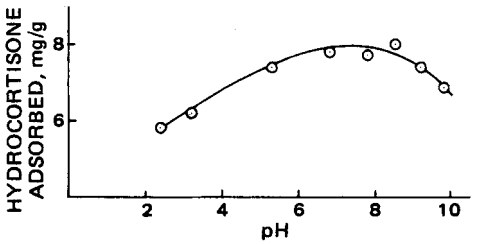

Figure 5—*Effect of pH on adsorption of hydrocortisone by sepiolite.*

supports a weak adsorption mechanism. Generally, adsorption due to hydrogen bonding can be detected by shifts in the absorption bands of participating functional groups. Some hydroxyl groups of hydrocortisone might be expected to take part in hydrogen bonding with the clay surface. Evidence for hydrogen bonding is seen in the shift of the hydroxyl stretching vibrations of hydrocortisone at 3420 and 3285 cm^{-1} to 3400 and 3260 cm^{-1} in the presence of sepiolite. In addition, the carbonyl stretching vibration of the C-17 hydrocortisone side chain was shifted from 1700 to 1655 cm^{-1} as a result of interaction with sepiolite. The shift of these groups to a lower frequency suggests that hydrogen bonding is the principal adsorption mechanism, although van der Waals forces also may contribute. The fact that the CH-stretching vibrations at 2960, 2920, and 2860 cm^{-1} were not shifted in the presence of sepiolite suggests that the energy of interaction with sepiolite is similar to the intermolecular bonding in hydrocortisone. The IR spectrum clearly shows significant adsorption of hydrocortisone by sepiolite. This finding also is in sharp contrast to the interaction of hydrocortisone with attapulgite, where no change in the IR spectrum was observed after interaction with hydrocortisone (1).

To evaluate the amount of hydrocortisone adsorbed by sepiolite, the adsorption isotherm at pH 8.4 was determined (Fig. 4). The isotherm is of the L or Langmuir type according to the classification of Giles *et al.* (14), who, along with Knight and Tomlinson (15), suggested that this type of isotherm indicates a specific interaction between the adsorbate and the adsorption sites. The adsorption data were applied to both the theoretical Langmuir isotherm equation and the empirical Freundlich equation. The adsorption data gave a good linear fit with both the Langmuir and Freundlich isothermal plots (r = 0.9901 and 0.9937, respectively). The maximum adsorption of hydrocortisone by sepiolite as determined by the slope of the Langmuir plot was 8.75 mg/g.

The adsorption of hydrocortisone by sepiolite is pH dependent (Fig. 5). Maximum adsorption occurred between pH 7 and 8.5. It is hypothesized that the surface Si-OH groups of sepiolite are uncharged in this pH range. Thus, interaction occurs most strongly between the neutral hydrocortisone molecule and the neutral sepiolite surface.

Desorption studies also confirmed that hydrocortisone is adsorbed weakly by sepiolite since it was desorbed readily by washing with water at pH 2.9, 6.8, or 9.8 (Table II).

The rheograms of 1 and 2% sepiolite suspensions are very similar to the rheograms of 1 and 2% attapulgite suspensions, suggesting that sepiolite could be used in place of attapulgite as a pharmaceutical excipient (Fig. 6).

The minimal effect of sepiolite on the oxidative degradation of hydrocortisone in comparison to the catalytic effect of attapulgite is consistent with the small amount of iron (0.3%) present in the sepiolite

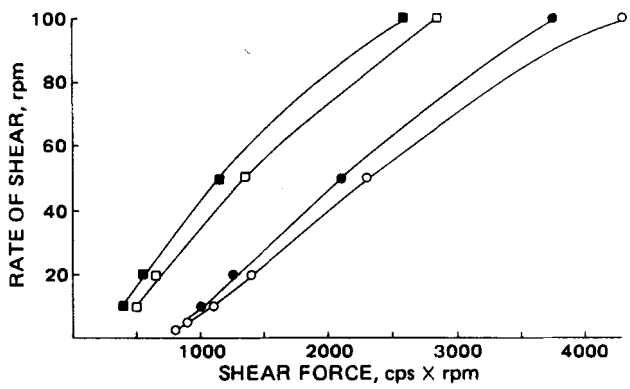

Figure 6—*Rheograms of sepiolite and attapulgite suspensions. Key:* ■, *1% sepiolite;* □, *2% sepiolite;* ●, *1% attapulgite; and* ○, *2% attapulgite.*

Table II—Desorption from Hydrocortisone–Sepiolite Suspensions by Washing with Water

pH	Hydrocortisone Adsorbed, mg/g	Hydrocortisone Desorbed, mg/g		
		First Wash	Second Wash	Third Wash
2.9	5.75	1.03	0.63	0.60
6.8	7.91	3.17	1.45	0.86
9.8	6.69	1.50	0.88	0.80

sample compared to the iron content of the attapulgite studied previously (2.6%). The oxidative degradation of hydrocortisone is affected minimally by sepiolite, although significant adsorption occurs. Adsorption of hydrocortisone by sepiolite can be explained by the high external surface area of sepiolite (400 m^2/g). However, attapulgite also has a significant external surface area (280 m^2/g), but no adsorption of hydrocortisone by attapulgite was observed by IR spectroscopy (1). It is hypothesized that the contact time needed for adsorption is greater than the contact time needed for the ferric iron-catalyzed oxidative degradation. Thus, in clays with a high ferric iron content, oxidative degradation is the predominant reaction. However, in clays such as sepiolite with a low ferric iron content and, therefore, a much smaller catalytic effect, the major reaction is adsorption.

The results of this study strongly suggest that sepiolite should be considered for use in pharmaceuticals. It has a very similar structure to attapulgite but a greater external surface area, which suggests that sepiolite will have excellent properties as a GI adsorbent. In addition, sepiolite is a desirable clay for use as a pharmaceutical excipient since its low ferric iron content means that it is compatible with drugs such as hydrocortisone that degrade by oxidative degradation. In addition, the reversible nature of the adsorption of hydrocortisone by sepiolite suggests that the bioavailability of neutral drugs will not be affected significantly by interaction with sepiolite. Finally, the rheological properties of sepiolite suspensions are very similar to attapulgite suspensions.

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Synthesis and Anticonvulsant Activity of Racemic 2-Amino-*N*-substituted Succinimide Derivatives

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Received June 16, 1980, from the *College of Pharmacy, University of Toledo, Toledo, OH 43606.* Accepted for publication July 25, 1980.

Abstract □ Several derivatives of (*R,S*)-2-amino-*N*-substituted succinimides were synthesized and evaluated in mice against seizures produced by electroshock and pentylenetetrazol. The most active compound against both electroshock- and pentylenetetrazol-induced seizures was (*R,S*)-*N*-benzyl-2-(methanesulfamido)succinimide.

Keyphrases □ 2-Amino-*N*-substituted succinimide derivatives—synthesis and evaluation for anticonvulsant activity □ Anticonvulsant activity—2-amino-*N*-substituted succinimide derivatives, synthesis and evaluation for activity □ Structure–activity relationships—2-amino-*N*-substituted succinimide derivatives, synthesis and evaluation for anticonvulsant activity

Many epileptic seizures cannot be controlled by currently available anticonvulsants. Furthermore, those individuals whose seizures are controlled often tolerate harmful side effects (1). The development of carbamazepine and valproic acid has improved seizure protection for epileptics, of whom only 50% were completely protected by previously marketed antiepileptic drugs. Despite the beneficial effects of these drugs, new anticonvulsants with more selective action and less toxicity are needed (2).

BACKGROUND

In the development of new anticonvulsants, most attention has been centered on the ureide structure (I). Three succinimides, phensuximide, methsuximide, and ethosuximide, that contain this basic structure are used in the treatment of petit mal epilepsy (3).

Witiak *et al.* (4) reported the synthesis and anticonvulsant activity of

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