

# Identification of a pterin as the acrasin of the cellular slime mold *Dictyostelium lacteum*

(chemotaxis/cell aggregation/pterin deaminase/pterin derivatives/HPLC)

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**ABSTRACT** Cell aggregation in *Dictyostelium discoideum* is mediated by chemotaxis to cyclic AMP. Aggregative cells of the simpler species *D. lacteum* are not attracted by this cyclic nucleotide. We describe how the cell aggregation-inducing factor, or acrasin, of *D. lacteum* was purified from aggregating amoebae and characterized. The acrasin, which is mainly secreted in the aggregative phase, is identified as a derivative of pterin. This identification is based on (i) its UV spectrum, (ii) the inhibition of the enzymatic degradation of acrasin by 6-methylpterin, (iii) the antagonistic effect of 6-aminopterins on chemotaxis towards both pterin and acrasin and not on the response towards folic acid or cyclic AMP, and (iv) the degradation of the acrasin to pterin. Its chromatographic properties show that the acrasin is an as yet unidentified pterin derivative. The acrasin is species specific and attracts cells at very low concentrations (0.1–0.01  $\mu$ M). Also, several naturally occurring stereoisomers of 6-polyhydroxyalkylpterins attract aggregative cells at these low concentrations. Additionally, we identified a pterin deaminase, which converts pterin into 2-deamino-2-hydroxypterin (lumazin), as the acrasinase in *D. lacteum*.

Free-living cells of the cellular slime molds feed on bacteria. Vegetative cells of all species so far tested are attracted by folic acid, which is chemotactically less active in later stages (1). Lack of food induces amoebae to aggregate. Cell aggregation is mediated by a chemoattractant or acrasin (2). Cyclic AMP, which was the first identified acrasin, induces cell aggregation in *Dictyostelium discoideum* (3–5) and attracts other *Dictyostelium* species with large fruiting bodies (6). These species are characterized by autonomous oscillations and a relay of the cyclic AMP signal from the center outward. The outward movement of the chemotactic signal coincides with wave-like pulsations of cells moving inward. The amoebae form intercellular contacts and condense into streams of cells moving into the aggregate center. In *D. discoideum* it has been shown that these behavioral changes are accompanied by an increase in cyclic AMP binding sites, enzyme activities, and cell surface glycoproteins (for a review see ref. 7). Besides having a chemotactic effect during cell aggregation, cyclic AMP induces cell differentiation (8).

We wished to investigate the molecular mechanism of signal transfer in a less complex cell aggregation system. *D. lacteum* was chosen for its simple type of aggregation without pulsations or streams. To investigate signal transfer in this species the acrasin should be characterized to such an extent that the attractant or an active derivative can be used as a signal molecule.

Recently, unidentified attractants of *Polysphondylium violaceum* (9), *D. minutum* (10), and *D. lacteum* (11) have been purified and characterized. At least five compounds that are

active in attracting cells of *D. lacteum* have been isolated from yeast extract (unpublished results). To be certain that any isolated attractant was the acrasin of *D. lacteum*, secretion products of aggregating cells were used as the source for this investigation. We describe the identification of the *D. lacteum* acrasin as a pterin derivative and suggest some commercially available, just as active, analogues to be used for further research.

## MATERIALS AND METHODS

**Organisms and Culture Conditions.** *Dictyostelium discoideum* NC-4(H) was cultivated on SM-agar (12) and *D. lacteum*, *D. minutum*, *D. vinaceo-fuscum*, and *Polysphondylium violaceum* on 0.1% lactose/peptone agar together with *Escherichia coli* B/r. Cells were harvested in the late logarithmic phase and washed in 1% standard salt solution (2) three times by centrifugation at  $150 \times g$  for 4 min.

**Chemotaxis.** We used the small population assay to measure chemotactic activity (13). This involves the deposition on hydrophobic agar of two small, closely spaced droplets, one containing sensitive cells and the other a test substance. The chemotactic response is considered positive when more than twice as many cells are pressed against the margin closest to the attracting drop as against the opposite side. Preparations were neutralized before testing, if necessary.

**Acrasin Isolation.** A dialysis tank containing a 1% standard salt solution (14) was used to isolate acrasin from approximately  $2 \times 10^8$  aggregating amoebae. In this way the secreted attractant was separated from degrading enzymes. The medium was replaced before and after aggregation and concentrated by evaporation under low pressure at 45°C.

**Acrasinase Isolation.** Cells of *D. lacteum* were shaken in 0.01 M sodium/potassium phosphate buffer, pH 6.0, on a New Brunswick shaker (150 rpm) at a cell density of  $10^7$  per ml. After 3 hr the suspension was centrifuged at  $150 \times g$  for 4 min and the supernatant was centrifuged for 10 min at  $20,000 \times g$ . The acrasinase in the resulting supernatant remained active during storage at  $-20^\circ\text{C}$  for several months.

**Enzymatic Degradation of Pterin Derivatives.** Pterin derivatives were incubated at a final concentration of 0.1 mM with different amounts of the acrasinase preparation. Incubations at 20°C were terminated and analyzed after the desired time intervals (up to 24 hr) by injection of a sample into HPLC system G (see below). In the case of reduced pterins (dihydro and tetrahydro compounds) 2 mM dithiothreitol (Sigma) was included in the incubation mixture and in the mobile phase to exclude degradation due to oxidation; enzymatic degradation was analyzed for 1 hr. Dithiothreitol has no effect on the degradation velocity of pterin or the acrasin. When no degradation was observed on HPLC this was verified by recording UV spectra during the incubation on a Beckman 3600 spectrophotometer.

**HPLC and Mobile Phase Composition.** The HPLC equip-

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ment consisted of a Beckman 100 A pump and a Laboratory Data Control UV III (1203) monitor, set at 254 nm. Reversed-phase Lichrosorb 5 RP 18 (Merck), cation exchanger Partisil PXS 10/25 SCX (Whatman), and anion exchanger Partisil PXS 10/25 SAX columns were used. The flow rate was 1 ml min<sup>-1</sup>.

Mobile phase compositions: A, 15 mM sodium phosphate buffer, pH 6.0/10% (vol/vol) methanol (for RP 18). B, 10 mM NH<sub>3</sub>, solution adjusted with CH<sub>3</sub>COOH to pH 4.0 (for SCX). C, 10 mM NaH<sub>2</sub>PO<sub>4</sub>/20% methanol, adjusted with H<sub>3</sub>PO<sub>4</sub> to pH 2.0 (for SCX). D, 15 mM NaH<sub>2</sub>PO<sub>4</sub>/20% methanol, adjusted with H<sub>3</sub>PO<sub>4</sub> to pH 2.0 (for SCX). E, 10% methanol in H<sub>2</sub>O (pH 6.0, without salts added) (for RP 18). F, 0.1 M NH<sub>3</sub>/0.2 M NaCl/20% (vol/vol) 1-propanol/10% (vol/vol) CH<sub>3</sub>CN, adjusted with CH<sub>3</sub>COOH to pH 5.3 (for SAX). G, 85 mM NaH<sub>2</sub>PO<sub>4</sub>/15% methanol, adjusted to pH 2.0 with H<sub>3</sub>PO<sub>4</sub> (for SCX).

**Permanganate Oxidation.** One microliter of 0.5 mM KMnO<sub>4</sub> in 0.1 M NaOH was added to 2 μl of 5 μM acrasin. The sample was boiled and alkaline permanganate was added slowly until the color ceased to disappear. Boiling was extended for 15 min, and excess KMnO<sub>4</sub> was removed by the addition of methanol. After centrifugation and neutralization the supernatant was injected in HPLC system F. (Pterin-6-carboxylic acid elutes at  $k' = 1.0$  and folic acid at  $k' = 2.25$ .  $k'$  is column capacity ratio.) D-erythro-Neopterin and 6-hydroxymethylpterin were processed in an identical way.

**Chemicals.** Compounds 1, 2, 17, 18, 22, and 23 in Table 2 were purchased from Sigma; 16, 19, 24, and 29 were from Fluka; and 21 was from Serva (Heidelberg, Federal Republic of Germany). T. Sugimoto (Nagoya University, Japan) generously supplied compounds 9–13 and 30; W. Pfeleiderer (University of Konstanz, Federal Republic of Germany) supplied 3–7 and 20; and compounds 14 and 15 were a gift from M. Viscontini (University of Zürich). 6-Aminopterin was synthesized as reported (15) and characterized by its UV spectrum after purification by HPLC. Sepiapterin was synthesized as described in ref. 16, and 7,8-dihydropterin, 7,8-dihydro-L-erythro-biopterin, and 7,8-dihydro-D-erythro-neopterin were synthesized by reduction of the parent compounds according to ref. 17 and purified by HPLC.

Table 1. Chromatographic data for chemotactic activities and UV peaks of the purified products from aggregative *D. lacteum* cells

Compound	$k'$ values with system					
	A	B	C	D	E	F
a	0.4–0.7	2.5–3.1	3.8–4.4	0.8–1.3	1.9–3.4	ND
b	1.2–1.6	0.9–1.3	2.0–3.0	0.8–1.3	1.2–1.6	ND
c	0.55	2.8	4.1	1.0	2.7	0.19
d	0.52	2.7	2.8	0.6	2.6	0.19
e	1.32	1.03	2.4	0.9	1.4	0.15

Purified products of *D. lacteum* cells were injected into six HPLC systems. The eluate was monitored for chemotactic activity and for UV absorbance. Detected: a, major chemotactically active material; b, minor chemotactically active material; c, UV peak of pure major chemotactically active material; d, UV peak of enzymatic degradation product; e, UV peak of chemical degradation product.  $k'$  values were calculated with  $k' = (V_r - V_0)V_0^{-1}$ , in which  $V_r$  is the retention volume of the compounds and  $V_0$  is the dead volume of the column. ND, not determined because of the high salt concentration of the mobile phase.

## RESULTS

**Purification of Chemotactic Compounds.** Postvegetative cells did not secrete a measurable amount of chemoattractant until 2 hr before aggregation. The dialyzed secretion product of aggregating cells was concentrated and analyzed by HPLC. All systems except D resulted in clear separation of two active compounds, of which one was about 10-fold more active than the other (Table 1). Steps in the purification of the major activity fraction are shown in Fig. 1. After purification with system D, this active compound was chromatographically pure as judged by reinjection and chromatography with systems A–F. The chromatograms from systems E and B are shown in Fig. 1 D and E.

**Enzymatic Degradation of the Major Activity Fraction.** The highly purified major activity fraction was incubated with an acrasinase preparation. The chemotactic activity disappeared, and HPLC system D revealed one UV-absorbing product corresponding to the peak at 5.2 ml in Fig. 1C. With systems A, B, E, and F the enzymatic degradation product eluted together with the major activity, indicating a close resemblance of the active substance and its enzymatically converted inactive prod-

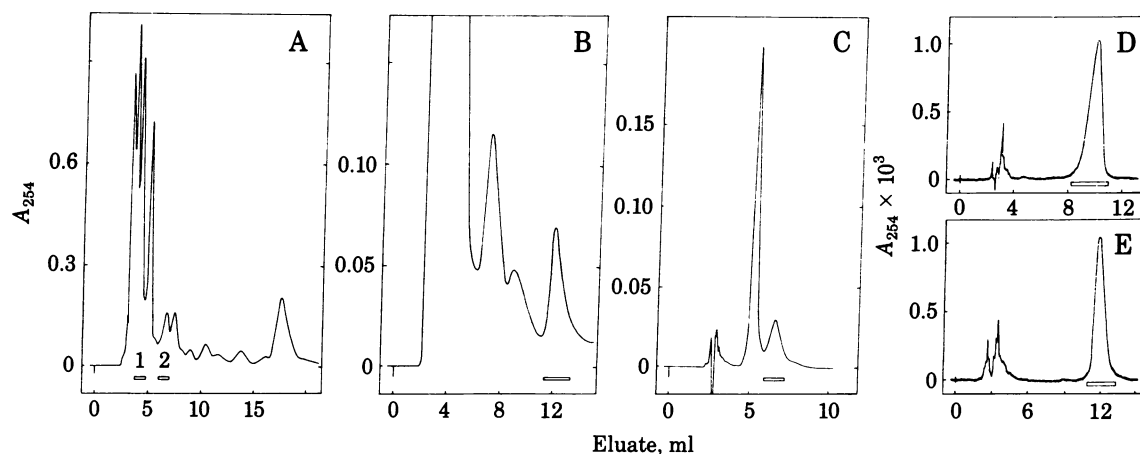


FIG. 1. Purification of the major chemotactically active material. (A) The secretion products of  $4 \times 10^8$  aggregative *D. lacteum* cells were chromatographed with HPLC system A. Fractions of the eluate were concentrated to 60 μl and tested for chemotactic activity. 1, Major activity; 2, minor activity. (B) Fraction 1 from A was chromatographed with HPLC system B. The bar represents the active fraction. (C) The active material from B was separated with HPLC system D. Before chemotaxis was tested, fractions were concentrated by evaporation under low pressure at 45°C. Mobile phase D was neutralized with Ca(OH)<sub>2</sub> and the precipitate was centrifuged and washed once. The combined supernatants were concentrated and tested for chemotactic activity. (D) Chromatogram of the purified major activity with system E. (E) Rechromatography of the purified major activity with system B.

uct. The only distinct difference was the positive charge at pH 2.0 (systems C and D).

**Chemical Conversion of the Major Activity Fraction.** After prolonged storage at  $-20^{\circ}\text{C}$  in the dark, the pure major activity preparation was degraded. Analysis on HPLC revealed one UV-absorbing product; its  $k'$  values are listed in Table 1. Unexpectedly, this product was still chemotactically active. Boiling at neutral pH for 1 hr did not accelerate degradation, nor did acid conditions at  $20^{\circ}\text{C}$  at pH higher than 2. Boiling at pH 1 or 13 for 1 hr resulted in conversion to the same product as found after storage for several weeks. It is interesting that this product corresponds to the minor activity fraction in the unpurified secreted material as judged by HPLC on all systems (Table 1). Therefore, we conclude that the major activity is identical to the acrasin, and the minor activity corresponds to the product of its chemical conversion.

**Species Specificity.** The pure *D. lacteum* acrasin at a concentration of approximately  $5\ \mu\text{M}$ , as estimated from the UV spectrum (see below), was chemotactically inactive in *D. discoideum*, *D. minutum*, *D. vinaceo-fuscum*, and *P. violaceum*, all tested in the aggregative sensitive phase. The threshold for chemotaxis in *D. lacteum* just before the onset of aggregation was  $0.1\text{--}0.01\ \mu\text{M}$ . In postvegetative cells the attractant was less active ( $10\text{--}1\ \mu\text{M}$ ).

**UV Spectra.** The UV spectrum of the pure acrasin and its chemical degradation product are given in Fig. 2A. Due to the small amount of the active product the spectrum is probably not exact at wavelengths below 300 nm. Therefore, spectra of the enzymatic degradation product were measured at several pH values (Fig. 2B), because this compound was isolated in 5-fold higher amounts from the amoebae. The absorption maxima between 300 and 400 nm in all these spectra led us to examine pterin derivatives and folates as candidates for the acrasin. A normal extinction coefficient at  $\lambda_{\text{max}} = 380\ \text{nm}$  at pH 5.0 for pterin derivatives is  $6 \times 10^3\ \text{M}^{-1}\ \text{cm}^{-1}$  (18). This value was used to estimate the concentration of pure acrasin.

**Pterin Derivatives Compete with the Acrasin for Degrading Enzyme(s) and the Chemotactic Receptor.** Fig. 3 shows that the enzymatic degradations of the acrasin and 6-methylpterin have the same velocity. If both compounds are degraded by the same enzyme, then an excess of 6-methylpterin should inhibit the degradation of the acrasin. About  $0.5\ \mu\text{M}$  acrasin and

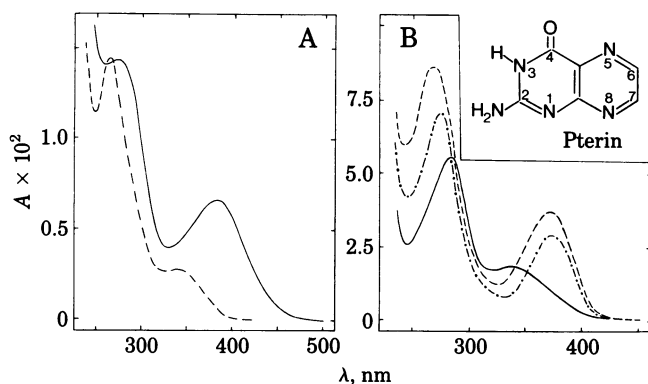


FIG. 2. UV spectra of compounds purified from the secretion products of aggregating amoebae. (A) —, Acrasin at pH 5.0 after purification with systems A, B, D, and E.  $\lambda_{\text{max}}$  occur at about 275 and 381 nm. ---, Chemical degradation product of acrasin separated from nondegraded acrasin with system E.  $\lambda_{\text{max}}$  are 265 and 340 nm at pH 5.0. (B) Enzymatically degraded acrasin purified with systems A, B, D, and E. —, pH 1.0,  $\lambda_{\text{max}}$  284 and 340 nm; ---, pH 5.0,  $\lambda_{\text{max}}$  269 and 371 nm; ···· pH 13.0,  $\lambda_{\text{max}}$  274 and 373 nm. (Inset) Numbering of atoms in pterin molecule.

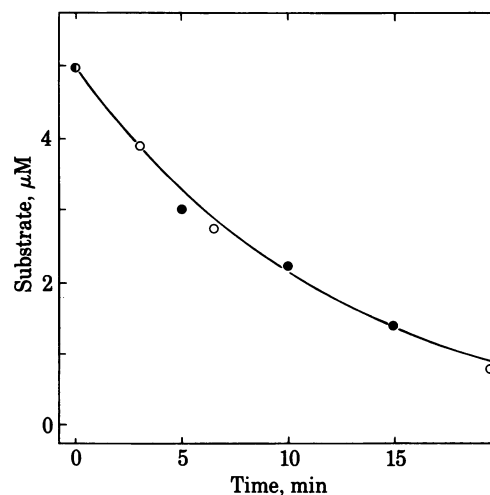


FIG. 3. Degradation of acrasin (●) and 6-methylpterin (○). Three microliters of acrasin ( $10\ \mu\text{M}$ ) or 6-methylpterin ( $10\ \mu\text{M}$ ) was incubated with  $3\ \mu\text{l}$  of acrasinase (diluted 1:100 in 10 mM phosphate buffer, pH 6.0). At the times indicated  $1\text{-}\mu\text{l}$  samples were injected into HPLC system D. Degradation has been calculated by measuring the peak areas of product and substrate in the chromatograms.

acrasinase were incubated with and without  $0.5\ \text{mM}$  6-methylpterin for 10 min and then acrasin and 6-methylpterin were separated with system A. A control experiment revealed that 6-methylpterin did not result in chemotactic activity in the acrasin fraction. The results revealed that without addition of 6-methylpterin the chemotactic activity of the acrasin was lost completely, whereas in the presence of 6-methylpterin no significant degradation was observed. The same was true when, instead of the acrasin, its chemical degradation product or the minor activity fraction was incubated with acrasinase and 6-methylpterin.

Another approach was to use 6-aminopterin, which is chemotactically inactive. This compound is an antagonist of chemotactically active pterin derivatives, but not of folic acid or cyclic AMP (19). Thus, a 10- to 100-fold excess of 6-aminopterin can block the chemotactic response of *D. lacteum* towards an active pterin analogue. We observed that the acrasin, its chemical degradation product, and the minor activity compound were antagonized by  $10\ \mu\text{M}$  6-aminopterin.

**Chromatographic Data of Pterin Derivatives.** The  $k'$  values of a series of pterin derivatives in three separation systems are shown in Table 2. Comparison of data in Tables 1 and 2 led us to the conclusion that none of these compounds corresponds to the acrasin or its enzymatic degradation product. The chemical degradation product, however, could be identified as pterin, not only by  $k'$  values, but also by the UV spectrum (Fig. 2A) and its chemotactic activity in *D. lacteum*. This confirms the evidence that the acrasin is a pterin analogue.

**Enzymatic Conversion and Chemotactic Activity of Pterin Derivatives.** Because the chromatographic properties of the pterin derivatives gave no clue to further identification of the acrasin, we tried another approach. The chemotactic activity of all analogues was studied to map the receptor specificity, and the degradation velocity of these analogues by the acrasinase preparation was studied to provide information about the specificity of the acrasinase(s). The only enzymatic reaction that occurred with pterin was deamination at the C-2 position, and 2-deamino-2-hydroxypterin (lumazin) was the only product. Also, for all other pterin derivatives that were degraded, only a disappearance of a positive charge at pH 2.0 could be observed. This strongly suggests that the pterin derivatives are degraded

Table 2. Chromatographic data and biological activities of pterin derivatives

Compound	<i>k'</i> values with system			Threshold activity, M					No.	Enzymatic degradation rate				
	No.	A	B	C	10 <sup>-8</sup>	10 <sup>-7</sup>	10 <sup>-6</sup>	10 <sup>-5</sup>		10 <sup>-4</sup>	10 <sup>-3</sup>	10 <sup>-2</sup>	10 <sup>-1</sup>	1
Acrasin	—	0.55	2.8	4.1						1				
Pterin	1	1.32	1.03	2.35						1				
Lumazin	2	1.14	0.44	0.15						2				
N <sup>2</sup> -Methylpterin	3	3.47	1.59	2.15						3				
N <sup>2</sup> -Dimethylpterin	4	6.36	3.87	2.88						4				
N <sup>3</sup> -Methylpterin	5	3.27	1.47	2.15						5				
2,4-Diaminopteridin	6	4.61	>6.75	4.98						6				
6-Methylpterin	7	3.45	2.35	3.74						7				
6-Aminopterin	8	1.07	1.89	2.79						8				
6-Hydroxymethylpterin	9	1.29	0.40	1.55						9				
L-erythro-Biopterin	10	0.98	0.30	1.16						10				
D-erythro-Biopterin	11	0.98	0.30	1.16						11				
L-threo-Biopterin	12	1.28	0.37	1.30						12				
D-threo-Biopterin	13	1.28	0.37	1.30						13				
L-erythro-Neopterin	14	0.36	0.20	1.01						14				
D-erythro-Neopterin	15	0.36	0.20	1.01						15				
L-threo-Neopterin	16	0.41	0.22	1.09						16				
Pterin-6-carboxylic acid	17	0.25	-0.16	0.60						17				
6-Hydroxypterin	18	1.18	0.23	0.42						18				
Folic acid	19	5.64	11.00	2.25						19				
7-Methylpterin	20	3.41	2.81	4.26						20				
Pterin-7-carboxylic acid	21	0.25	-0.18	0.54						21				
7-Hydroxypterin	22	1.12	0.20	0.11						22				
6,7-Dihydroxypterin	23	0.73	0.10	0.06						23				
5,6,7,8-Tetrahydrofolic acid	24	0.74	>11.0	3.87						24				
7,8-Dihydro-L-erythro-biopterin	25	0.92	0.57	1.63						25				
7,8-Dihydro-D-erythro-neopterin	26	0.38	0.34	1.37						26				
7,8-Dihydropterin	27	0.78	2.42	3.45						27				
Sepiapterin	28	0.88	ND	1.47						28				
5,6,7,8-Tetrahydropterin	29	0.59	10.60	8.12						29				
5,6,7,8-Tetrahydro-L-erythro-biopterin	30	0.53	4.89	4.08						30				

The chemotactic threshold activity was determined for aggregative cells; cross-hatched bars indicate the concentration range, in which the lower limit induced chemotaxis in less than 50% of the populations, the higher limit induced in more than 50%. The relative enzymatic degradation rate was measured by HPLC or UV absorption. The degradation rate of pterin was set at 1; empty bars represent the initial velocity of degradation related to pterin. For several analogues no degradation was observed. The upper limit for the rate of degradation of these analogues is given by the hatched bars. ND, not determined; i, inactive.

\* This value is based on the observation that the velocity of degradation is similar for the acrasin, 6-methylpterin, and pterin, all at 5  $\mu$ M.

enzymatically by a hydrolytic deamination at the C-2 position. Combining these data with the results shown in Fig. 3, we propose that the acrasin is enzymatically degraded by a pterin deaminase.

A comparison of the structures of pterin derivatives and their chemotactic activities and degradation velocities (Table 2) shows that reduced pterins (compounds 23–30) are unlikely candidates for the acrasin, because they are degraded less than 1/100th as fast as the acrasin. Furthermore, modifications in the pyrimidine ring are not allowed (compounds 2–6). The pyrazine ring, however, possesses one atom, C-6, at which substituents are permitted. In several cases 6-hydroxyalkyl derivatives show chemotactic activity equal to that of the acrasin.

**Chemical and Physical Properties.** A chemical test for 6-hydroxyalkylpterins is alkaline permanganate oxidation (18, 20), which should yield pterin-6-carboxylic acid. Oxidation of the acrasin did not yield pterin-6-carboxylic acid under conditions that resulted in formation of pterin-6-carboxylic acid from 6-hydroxymethylpterin or D-erythro-neopterin. Analysis with HPLC system F revealed another, more negatively charged, compound. This finding does not support or exclude the possibility that the acrasin is a 6-alkylpterin derivative.

$pK_a$  measurements were performed by HPLC cation exchange (21). In the pH range of 1.5–7.0 the acrasin had two  $pK_a$

values, 3.3 and 1.0, whereas the enzymatic degradation product had only one  $pK_a$ , 3.2. Because the only difference in molecular structure is the C-2 amino substituent, the  $pK_a$  of 1.0 should represent the basic N-1 atom (19). In the pH range of 1.5–7.0 no other  $pK_a$  was reported in the pterin moiety (19), thus the  $pK_a$  of 3.3 might reflect a functional group in the C-6 position.

We observed a very ion-sensitive behavior of the acrasin on

Table 3. Effect of counterions on the polarity of charged and noncharged compounds

Compound	<i>k'</i> values with system		Ratio E/A
	A	E	
Acrasin	0.55	2.7	4.91
Pterin	1.32	1.4	1.06
D-erythro-Neopterin	0.36	0.38	1.05
8-Aminobutylamino-cAMP	6.20	12.5	2.02

Retardation of the acrasin and several other compounds on a reversed-phase column was monitored with mobile phase systems A and E. These two systems differ in ion concentration: A contains 15 mM phosphate buffer, pH 6.0, whereas E consists of distilled water without salts added (pH 6.0). To both systems 10% methanol was added. The ratio between *k'* values on system E and those on system A reflects the effect of counterions on the polarity of these compounds.

a reversed-phase column at pH 6 (Table 3). This effect of buffer ions on the polarity is also observed with zwitterions (e.g., 8-aminobutylamino-cyclic AMP) but not with noncharged apolar compounds (pterin) or noncharged polar compounds (*D-erythro*-neopterin). This is due to ion-pair formation in the mobile phase (22). Such ion pairs are not formed with the noncharged compounds. Therefore, we conclude that the acrasin is charged at pH 6.

However, we observed low  $k'$  values of the acrasin at pH 5–6 on the cation exchanger (data not shown) and on the anion exchanger, system F, which indicates the absence of a net charge in the acrasin molecule at pH 6. Thus, the acrasin probably is a zwitterion, because it is charged but without a net charge at pH 6. As a consequence of the acrasin being a zwitterion at pH 6.0, the  $pK_a$  of 3.3 should reflect an acidic function, i.e., negative charge. The  $pK_a$  of the positive charge should be above pH 7.0.

### DISCUSSION

A complicating factor during the purification and characterization of the *D. lacteum* acrasin was the low yield. One isolation experiment yielded 0.1 nmol or less acrasin and about 0.5 nmol of the enzymatically deaminated form; therefore, it was not possible to apply structure identification techniques, such as NMR. Four independent lines of evidence have been presented which show that the *D. lacteum* acrasin is a pterin derivative: UV spectra, competition with 6-methylpterin for the acrasinase, 6-aminopterin antagonizing the chemotactic response to the acrasin, and the fact that chemical conversion of the acrasin yields pterin. Because the pure attractant showed properties incompatible with presently known natural derivatives and some chemically synthesized pterin analogues, we were unable to identify the entire chemical structure.

In summary, we assign the following properties to the acrasin: it consists of a pterin moiety with an unknown substituent, probably at C-6. An alkyl side chain with an amino and carboxyl function would be a suitable candidate for this substituent. Many 6-alkylpterins have a  $\lambda_{max}$  of 360 nm (18). The high  $\lambda_{max}$  of 380 for the acrasin suggests one double bond in the side chain, conjugated with the pterin  $\pi$ -electron system. In addition, stereospecific recognition of the side chain by the chemotaxis receptor is indicated by the high activity of *D-erythro*- and *L-threo*-6-polyhydroxyalkylpterins (neo- and biopterin).

The acrasinase has been characterized as a pterin deaminase, although the existence of two or more isozymes as in *D. discoideum* (23–25) cannot be excluded. The deaminase from *D. lacteum* shows the same substrate specificity as an enzyme isolated from *Alcaligenes metalcaligenes* (26), but is different from those of *D. discoideum* (23).

It is well known that many slime molds secrete folic acid (1); *D. lacteum* did not secrete a chemotactically detectable amount during aggregation, although a small amount was present (judged by HPLC). Until now, folic acid and pterins were regarded as compounds used in food seeking (1). With recognition of the acrasin of *D. lacteum* as a pterin, which is functional during the process of cell aggregation, another important action of

the pterins has been presented. The fact that food detection and cell aggregation involve the same or related signal compounds may indicate an evolutionary relationship between these two processes.

As indicated by the antagonism of 6-aminopterin, the acrasin and pterins act by the same chemotaxis receptor. Thus the pterin structure can be regarded as the functional part of the acrasin molecule. The application of highly active derivatives such as *D-erythro*-neopterin, *D-erythro*-biopterin, *L-threo*-neopterin, and *L-threo*-biopterin should lead to further elucidation of the process of chemotaxis and cell aggregation, and comparison with more studied organisms may give information on the universality of these processes.

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