Effect of agents connected with the adenylate cyclase system on low pressure indentation

Agent	Concentration of active part (%)	Decrease in indentation $\times 10^{-3}$ cm 1 h	2 h	3 h	5 h	6 h	7 h	10 h
N^6,O^2,-dibutyryl c-AMP (sodium salt)	0.1	NS	2.6 ± 1.0	3.5 ± 0.8	4.5 ± 0.8	6.8 ± 1.3	8.0 ± 1.2	5.0 ± 0.8
Isoproterenol (bitartarate)	0.1	5.0 ± 0.8	7.3 ± 1.0	7.3 ± 1.7	7.3 ± 1.2	–	5.2 ± 0.8	NS
Papaverine (hydrochloride)	5.0	4.5 ± 1.0	6.3 ± 1.0	5.8 ± 1.3	4.3 ± 1.0	NS	NS	
Adenosine	0.1	4.0 ± 0.5	4.2 ± 0.8	4.2 ± 0.8	4.0 ± 1.0	NS		
Terbutaline (sulfate)	0.3	2.7 ± 1.0	6.5 ± 1.2	4.7 ± 1.5	4.5 ± 1.7	–	4.0 ± 1.3	NS

NS = not significant at $p \leq 0.05$. Agents were freshly dissolved in distilled water. A premeasured 1 ml was self-applied in successive small doses over a 10-min period to the forehead skin of the volunteers. Each figure represents the mean ± SD from the mean of 16 experimental points in four volunteers.

was also tested at one-third of the concentrations shown in the table, and at this level all were ineffective.

We also measured the elastic recovery of the skin before and after the application of all the agents mentioned in this communication (for the method see Dikstein and Hartzshtark[1]. None of these agents had any influence on elastic recovery within the time scale of these experiments.

One can only speculate on the possible role of c-AMP. In our view, it stimulates the fibroblasts to synthetize hyaluronic acid[8]. We have shown, indeed, that hyaluronidase increases indentation[9]. On the other hand, β_2 receptors have been identified in the epidermis[10].

The idea that active emollients and moisturizers could work via a pharmacological route has already been suggested by Tronnier[11] and Idson[12]. Van Dorp[13] postulated that dry skin conditions could be treated by prostaglandins and essential fatty acids externally applied to the skin. Penneys et al.[14] showed that white petrolatum interferes with the metabolism of arachidonic acid in the skin.

In this communication, however, a physical parameter (compressibility – 'firmness') of the human skin in vivo has been shown to be dependent on a biochemical intermediate *and* its pharmacological manipulation.

It is hoped that our findings will contribute to the development of cosmetic or pharmaceutical preparations aimed at hindering the effects of aging on the human skin.

Added in proof: U.S. patent 3,978,213 (31.8.1976) deals with the cosmetic use of c-AMP and agents inhibiting c-AMP degradation.

1 Dikstein, S., and Hartzshtark, A., in: Bioengineering and the Skin, p. 45. Ed. R. Marks. M.T.P. Press, Lancaster 1981.
2 Hartzshtark, A., and Dikstein, S., Rev. pure appl. Pharmac. Sci. *3* (1982) 83.
3 Dikstein, S., and Hartzshtark, A., Artz. Kosmet. *12* (1982) 280.
4 Dikstein, S., and Hartzshtark, A., Artz. Kosmet. *13* (1983) 327.
5 Duell, E.A., Molec. Pharmac. *18* (1980) 49.
6 Das, N.S., Chowdary, T.N., Sobhanadri, C., and Rao, K.V., Br. J. Derm. *99* (1978) 197.
7 Stavinsky, M.A., Powell, J.A., Lang, P.G., Schork, A., Duell, E.A., and Voorhees, J.J., J. invest. Derm. *64* (1975) 124.
8 Castor, C.W., J. Lab. clin. Med. *85* (1975) 392.
9 Dikstein, S., and Hartzshtark, A., Bioengng Skin *4* (1982) 48.
10 Duell, E.A., Biochem. Pharmac. *29* (1980) 97.
11 Tronnier, H., Parf. Kosm. *56* (1975) 217.
12 Idson, B., Drug Cosm. Indust. *126* (1980) 40.
13 Van Dorp, D., Ann. N.Y. Acad. Sci. *180* (1971) 180.
14 Penneys, N.W., Eaglstein, W., and Ziboh, V., Br. J. Derm. *104* (1980) 257.

0014-4754/85/030378-02$1.50 + 0.20/0

Growth inhibitory, insecticidal and antifeedant effects of some antileukemic and cytotoxic quassinoids on two species of agricultural pests[1]

J.A. Klocke, M. Arisawa, S.S. Handa, A.D. Kinghorn, G.A. Cordell and N.R. Farnsworth

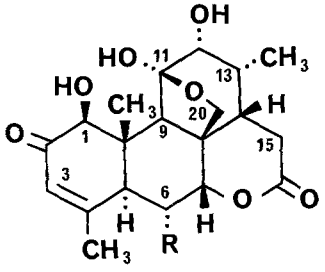
Plant Resources Institute, University Research Park, Salt Lake City (Utah 84108, USA) and Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, Health Sciences Center, University of Illinois at Chicago, Chicago (Illinois 60612, USA), 31 October 1983

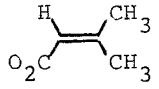
Summary. Several quassinoids, obtained by isolation and derivatization from *Simaba multiflora* and *Soulamea soulameoides*, were evaluated for growth inhibitory and insecticidal effects against the tobacco budworm *(Heliothis virescens)* and for antifeedant effects against *H. virescens* and the fall armyworm *(Spodoptera frugiperda)*. The relative activity of the quassinoids as insect growth inhibitors generally paralleled their known relative potency as antileukemic and cytotoxic agents.

Key words. Quassinoids; *Heliothis virescens*; *Spodoptera frugiperda*; growth inhibitory effects; antifeedant effects; antileukemic activity; cytotoxic activity.

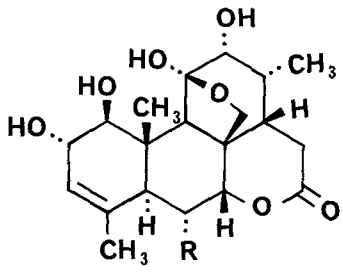
It is becoming increasingly evident that certain natural products elicit activity in a number of biological systems (e.g., antileukemic, insect antifeedant, antispomatic, insect antiecdysis, cytotoxic and brine shrimp toxic activities)[2–5]. For example, Nakanishi has pointed out that natural products with electrophilic moieties tend to be cytotoxic and insect antifeedant[6].

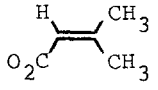
Such multiplicity in biological activities attributed to individual natural products has prompted us to investigate some plant products, previously isolated as anticancer agents, for their effects on pest insects. The present report describes the growth inhibitory, insecticidal and antifeedant effects of 8 quassinoids (simaroubolides) on the larvae of the economically important

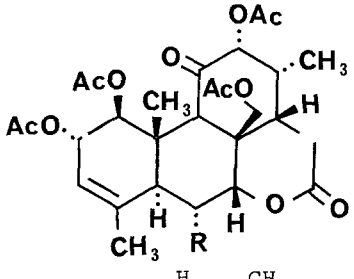


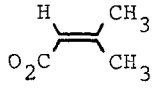
1 R = 

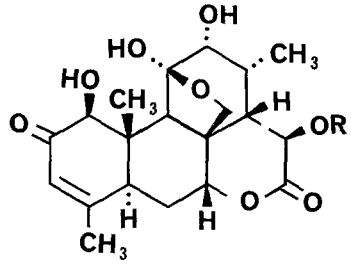
2 R = H



3 R = 

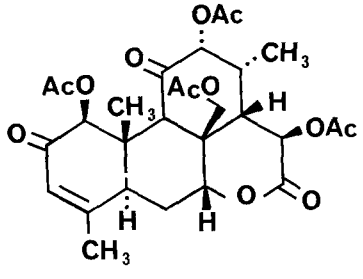


4 R = 

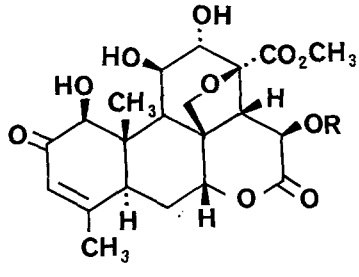


5 R = $COCH_3$

6 R = H



7



8 R = $COCH_2CH(CH_3)_2$

cotton budworm (*Heliothis virescens* Fabr.) and the fall armyworm (*Spodoptera frugiperda* J.E. Smith). Various quassinoids have previously been reported as anticancer[7–11], antiamoebic[12], antimalarial[13], antiviral[14] and locust insecticidal[15] agents.

A total of 6 quassinoids were isolated from the simaroubaceous species *Simaba multiflora* A. Juss. and *Soulamea soulameoides* (Gray) Nooteboom through bioactivity-guided chromatographic fractionation, namely, 6α-senecioyloxychaparrinone (**1**), chaparrinone (**2**), 6α-senecioyloxychaparrin (**3**), holacanthone (**5**), glaucarubolone (**6**) and isobrucein A (**8**)[10,11]. These compounds were active against the P-388 lymphocytic leukemia test system in vivo, and the Eagle's carcinoma of the nasopharynx (KB) test system in vitro, when tested according to standard protocols[16]. Compounds **1–3**, **5**, **6** and **8**, as well as 2 acetylated derivatives, 6α-senecioyloxychaparrin tetraacetate (**4**) and holacanthone triacetate (**7**), were examined for growth inhibitory and insecticidal effects using an artificial diet 'no choice' feeding bioassay[17,18]. Growth inhibition was determined as the percentage difference in larval wet weight between treated and control insects. Newly hatched larvae of *H. virescens* were used as the test organism, and the results obtained for the 8 test compounds are shown in table 1.

The relative growth inhibitory and insecticidal activities of the quassinoids, as depicted in table 1, reveal certain structure-activity relationships. For example, 6α-senecioyloxychaparrinone (**1**) was ca. 350-fold more active as an insect growth inhibitor than was 6α-senecioyloxychaparrin (**3**). Structurally, **1** and **3** differ only in the A-ring, the former bearing an α-ketol group (a potential alkylating center), and the latter a glycol functionality. An ester functionality at C-6 or C-15 also increased the activity against *H. virescens*. For example, **1** was approximately 12-fold more active than chaparrinone (**2**), and holacanthone (**5**) was approximately twice as active as glaucarubolone (**6**), as insect growth inhibitors. It has been hypothesized that the ester side-chain is important for transport of antitumor quassinoids into intact cells, and that partial unsaturation in the A-ring is required for the inhibition of protein synthesis[19]. However, it has also been reported that the structural requirement of the ester side-chain for antileukemic activity of quassinoids is not essential for their viral antitransforming activity[14].

Three of the most potent quassinoids tested in the artificial diet bioassay, 6α-senecioyloxychaparrinone (**1**), chaparrinone (**2**) and isobrucein A (**8**) were evaluated further in a bioassay for an antifeedant (feeding deterrent) effect. Antifeedant activ-

ity can be at least partly responsible for the growth inhibition, by influencing food ingestion and digestion, and was determined utilizing the cotton leaf 'choice' bioassay[18]. Third-instar larvae of *H. virescens* and *S. frugiperda* were used as test organisms for this purpose. The results are shown in table 2. Data obtained indicate that the ester side-chain is apparently not necessary for insect antifeedant activity, since compounds **1**, **2** and **8** are approximately of equal potency when tested in this manner. In the case of insect antifeedant activity, a transport phenomenon would not be needed for the quassinoids to act at the level of the peripheral sensilla[20].

A 3rd functionality which may be important in eliciting the activity of the quassinoids is the ring-C oxide bridge (comprising the hemiketal linkage between C-11 and C-20 in compounds **1–3** and **5** and **6**, and the ether bridge between C-13 and C-20 in compound **8**). For example, complete acetylation of **5** to produce holacanthone triacetate (**7**) caused scission of the hemiketal bridge, resulting in marked relief of strain in ring C and increased hydrophobicity[8,11]. The acetylated product **7** was more than 40-fold less active than **5** as an insect growth inhibitor (table 1).

For comparative purposes, previously published data[21] on azadirachtin is included in tables 1 and 2. Azadirachtin is one of the most potent plant-derived insecticide and antifeedant compounds so far discovered[21]. Thus, 6α-senecioyloxychaparrinone (**1**) and azadirachtin were found to be of equivalent potency as growth inhibitors for *H. virescens* larvae (table 1), although the latter compound was about 2.5 and 3.5 times, respectively, more active as an insecticide (table 1) and antifeedant (table 2) against this test organism. However, **1** was some 80-fold less active than azadirachtin as an antifeedant against *S. frugiperda* (table 2).

The relative activity of the quassinoids as insect growth inhibitors was shown to approximately parallel their activities as antineoplastic agents against the P-388 murine lymphocytic leukemia test system and as cytotoxic agents against cells derived from the human carcinoma of the nasopharynx (KB) (table 3)[8,10,11]. However, chaparrinone (**2**) and 6α-senecioyloxychaparrinone (**3**) were exceptions to this trend in exhibiting insect growth activity against *H. virescens* to degrees that represented a reversal of their known antileukemic potencies. If correlation of activity in insect assays and in anticancer assays occurs consistently with other classes of plant-derived compounds, then the possibility exist for 1. applying the inexpensive, simple, and reproducible insect assays as preliminary screens for anticancer agents, and 2. testing all agents found active in anticancer assays for insecticidal activity. The former possibility would fulfill a need for less expensive general bioassays for the detection of a broad spectrum of activities[2,3,22], the latter a need for new sources and models of insect control agents[23–25].

Table 1. Growth inhibitory and insecticidal activities of selected quassinoids against newly hatched larvae of *Heliothis virescens* in a 10-day artificial diet bioassay

Compound	ED_{50} (ppm)[a]	LD_{50} (ppm)[b]
6α-Senecioyloxychaparrinone (**1**)	0.7	7
Chaparrinone (**2**)	8.5	30
6α-Senecioyloxychaparrin (**3**)	250	c
6α-Senecioyloxychaparrin tetraacetate (**4**)	300	c
Holacanthone (**5**)	9	150
Glaucarubolone (**6**)	17	210
Holacanthone triacetate (**7**)	370	c
Isobrucein A (**8**)	2.4	40
Azadirachtin[d]	0.7	< 2[e]

[a] ED_{50} values, derived from log dose-probit lines fitted by eye, are the effective doses for 50% growth inhibition. [b] LD_{50} doses are the lethal doses for 50% death. [c] No deaths resulted from concentrations as high as 1000 ppm. [d] Data taken from the literature[21]. [e] 2 ppm is the LD_{95} dose for this compound that resulted in 95% death.

Table 2. Antifeedant activity of 3 quassinoids against 3rd-instar larvae of 2 insect species in a 24-h leaf disk 'choice' bioassay

Insect species	Quassinoid	PC_{95} (μg/disk)[a]
Heliothis virescens	6α-Senecioyloxychaparrinone (**1**)	15
	Chaparrinone (**2**)	15
	Isobrucein A (**8**)	15
	Azadirachtin[b]	6
Spodoptera frugiperda	6α-Senecioyloxychaparrinone (**1**)	8
	Chaparrinone (**2**)	6
	Isobrucein A (**8**)	8
	Azadirachtin[b]	0.1

[a] PC_{95} values are amounts of test compounds/l cm^2 leaf disk resulting in < 5% damage to untreated disks. [b] Data taken from the literature[21].

Table 3. Comparison of insect growth inhibitory, antileukemic[a] and cytotoxic[a] activities of 6 quassinoids

Compound	*H. virescens*[b] ED_{50} (ppm)	P-388 in vivo assay[c] Best T/C (%)	Dose (mg/kg)	KB assay[d] ED_{50} (μg/ml)
6α-Senecioyloxy-chaparrinone (**1**)	0.7	198	1.0	0.003
Isobrucein A (**8**)	2.4	155	1.0	0.003
Chaparrinone (**2**)	8.5	145	40.0	0.025
Holacanthone (**5**)	9	154	3.8	0.11
Glaucarubolone (**6**)	17	132	5.0	0.11
6α-Senecioyloxy-chaparrin (**3**)	250	160	0.75	0.03

[a] Data taken from the literature[8,10,11]. [b] Insect growth inhibitory data taken from table 1. [c] Antileukemic activity for mice implanted with P-388 lymphocytic leukemia. [d] Cytotoxicity against the Eagle's carcinoma of the nasopharynx test system.

1 Phytochemical aspects of this work were supported by Contract CM-97295 with the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Md. Insects were kindly supplied by the agency of the United States Department of Agriculture at Brownsville, TX.
Address reprint request to A.D. Kinghorn at Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood Street, Chicago, Ill 60612, USA.

2 Nakanishi, K., in: Insect biology in the future, p. 603. Eds M. Locke and D.S. Smith. Academic Press, New York 1980.
3 Nakanishi, K., J. nat. Prod. *45* (1982) 15.
4 Kubo, I., Klocke, J.A., Matsumoto, T., Kamikawa, T., in: IUPAC pesticide chemistry, human welfare and the environment, rational and biorational design of pesticides and growth regulators, p. 169. Ed. J. Miyamoto. Pergamon Press, Oxford 1982.
5 Ferrigni, N.R., and McLaughlin, J.L., in: Proc. 24th Annual Meeting of the American Society of Pharmacognosy, Oxford, Mississippi, 1983; abstr. No. 23.
6 Nakanishi, K., in: Proc. Joint Meeting of the American Society of Pharmacognosy and the Society for Economic Botany, Boston, Massachusetts, 1981; abstr. No. 2.
7 Cordell, G.A., and Farnsworth, N.R., Lloydia *40* (1977) 1, and references therein.
8 Wani, M.C., Taylor, H.L., Thompson, J.B., and Wall, M.E., Lloydia *41* (1978) 478.
9 Polonsky, J., Varon, Z., Moretti, C., Pettit, G.R., Herald, C.L., Rideout, J.A., Saha, S.B., and Khastgir, H.N., J. nat. Prod. *43* (1980) 503.
10 Arisawa, M., Kinghorn, A.D., Cordell, G.A., and Farnsworth, N.R., J. nat. Prod. *46* (1983) 218.
11 Handa, S.S., Kinghorn, A.D., Cordell, G.A., and Farnsworth, N.R., J. nat. Prod. *46* (1983) 359.

12 Geissman, T.A., A. Rev. Pharmac. *4* (1964) 305.
13 Trager, W., and Polonsky, J., Am. J. trop. Med. Hyg. *30* (1981) 531.
14 Pierre, A., Robert-Gero, M., Tempete, C., and Polonsky, J., Biochem. biophys. Res. Commun. *93* (1980) 675.
15 Odjo, A., Piart, J., Polonsky, J., and Roth, M., C.r. Acad. Sci. Paris *293* (1981) 241.
16 Geran, R.I., Greenberg, N.H., MacDonald, M.M., Schumacher, A.M., and Abbott, B.J., Cancer Chemother. Rep., Part 3, *3* (1972) 1.
17 Chan, B.G., Waiss, A.C., Jr, Stanley, W.L., and Goodban, A.E., J. econ. Ent. *71* (1978) 366.
18 Kubo, I., and Klocke, J.A., Experientia *38* (1982) 639.
19 Liao, L.-L., Kupchan, S.M., and Horwitz, S.B., Molec. Pharmac. *12* (1976) 167.
20 Schoonhoven, L.M., in: Natural pesticides from the neem tree (*Azadirachta indica* A. Juss.), p.105. Eds H. Schumutterer, K.R.S. Ascher and H. Rembold. Proc. First Int. Neem Conf., Germany Agency for Technical Cooperation, Rottach-Egern, W.Germany, 1980.
21 Kubo, I., and Klocke, J.A., Agric. Biol. Chem. *46* (1982) 1951.
22 Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., and McLaughlin, J.L., Planta med. *45* (1982) 31.
23 Djerassi, C., Shih-Coleman, C., and Diekman, J., Science *186* (1974) 596.
24 Menn, J.J., J. agric. Fd Chem. *28* (1980) 2.
25 Hedin, P.A., J. agric. Fd Chem. *30* (1982) 201.

0014-4754/85/030379-04$1.50 + 0.20/0

A new plot to estimate protein molecular weight by density gradient ultracentrifugation

J.L. Rendón and M. Calcagno

Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de Mexico (UNAM), Apartado Postal 70-159, 04510 Mexico, D.F. (Mexico), 3 February 1984

Summary. A plot of the logarithm of the molecular weight against the logarithm of the sedimenting distance is proposed for estimation of protein molecular weight. The proteins are separated in acrylamide-containing linear density gradients, polymerized and stained after centrifugation.
Key words. Protein molecular weight; molecular weight, protein; density gradient ultracentrifugation; ultracentrifugation, density gradient.

Sedimentation analysis in density gradients using the preparative ultracentrifuge is a standard method for protein characterization. This method allows the estimation of sedimentation coefficients and molecular weights of the samples by comparison with appropriate standards[1]. In a variant of the method, the sedimenting proteins are detected by means of the usual protein stains in acrylamide-containing density gradients, polymerized by photocatalysis after centrifugation[2].

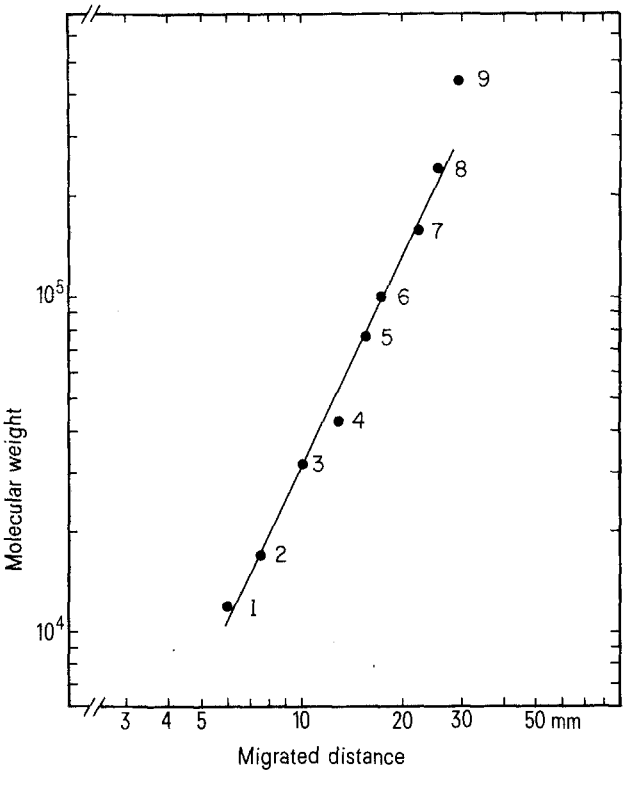
According to Martin and Ames, the position of the protein zones can be related to the corresponding sedimentation coefficients, which can be used to estimate molecular weights[1].

The plot that we propose is an alternative procedure for determining protein molecular weights, using linear sucrose density gradients in the presence or absence of acrylamide.

The acrylamide-containing density gradients, and the gel formed, were similar to the ones previously reported[3], with only slight modifications. Briefly, linear density gradients of sucrose from 5 to 30% (w/v) were formed in 5 ml cellulose nitrate tubes. These tubes contained 50 mM Tris-HCl buffer pH 8.8, 8% acrylamide, 0.3% N,N-methylene bis acrylamide, 0.2% N,N,N′,N′-tetramethylethylendiamine and 6 µg/ml riboflavine. Centrifugation was carried out in a Beckman L5-65 preparative ultracentrifuge with SW-50.1 rotor for 15 h at 300,000 × g. The tubes contents was polymerized by exposure to a long-wave UV source. The gels obtained were removed from the tubes by injecting water between gel and tube wall, and stained with Coomassie brillant blue G-250. The amount of protein detectable in each band under these conditions ranged from 10 to 100 µg. The sucrose density gradients without acrylamide monomers were also from 5 to 30% sucrose, in the same buffer.

The position of each band was measured directly with a millimeter rule from the top of the gel to the center of each band. The logarithm of molecular weight for each protein was plotted against the logarithm of the distance. In the case of conventional gradients without acrylamide, the abscissa was the logarithm of the number of the fractions collected counted from the starting radius.

As shown in the figure, a linear relationship was found for proteins from 12,000 to 240,000 daltons in acrylamide-contain-



Linear relation between the logarithm of the molecular weight and the logarithm of the migration distance for several proteins. Standards are: 1, horse heart cytochrome c (Mr 11,700); 2, horse muscle myoglobin (Mr 17,200); 3, half human hemoglobin (MR 32,000); 4, chicken egg albumin (Mr 44,600); 5, human transferrin (Mr 78,000); 6, yeast hexokinase (Mr 102,000); 7, rabbit muscle aldolase (Mr 160,000); 8, bovine liver catalase (Mr 232,000); 9, horse spleen ferritin (Mr 440,000). Each point represents the mean obtained from 12 independent experiments. The straight line was obtained by the method of least squares. (Excluding ferritin: r = 0.995.)