ticular oxidation product that is similar to b″. Allomerized in methanol or in barley leaves, b yields several pigments all more sorbed than b. One of these is spectrally similar to b (λ max. methanol, b, 652 mμ; oxidized b, 653 mμ). This product, therefore, resembles b″ both spectrally and chromatographically. Like b″ it is not isomerized by heat or alkalies; and it does not give the phase test. Except for the method of preparation, this allomerized b is identical with b″.

The a″, for which few properties have been determined,[3] has its counterpart among the oxidation products[2] of a. The increased sorbability of the oxidized chlorophylls[2] and of a″ and b″ relative to a and b may be attributed to additional oxygen (or decrease in hydrogen).[4]

Molecular structures proposed by Freed, *et al.*,[3] for the chlorophylls and their isomers are unsuitable. If a, a′, and oxidized a have the magnesium atom bound to different pairs of pyrrole nitrogen atoms,[3] the three pigments should yield but one magnesium-free pheophytin. Yet, three have been reported.[1,2] Moreover, oxidized chlorophylls, presumably with magnesium bound as in the chlorophylls, do not undergo isomerization.[2]

The strongly sorbed pigments prepared by Freed, *et al.*, and by Strain are oxidized chlorophylls, not isomeric chlorophylls. These products should not be called chlorophylls (unless found as natural constituents of plants).

(4) H. H. Strain, "Chromatographic Adsorption Analysis," Interscience Publishers, Inc., New York, N. Y., 1942, pp. 14–25.

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GLUTAMIC DEHYDROGENASE OF BEEF LIVER, A ZINC METALLOENZYME

Sir:

We recently have examined several crystalline preparations of beef liver glutamic dehydrogenase and find that zinc is an integral and functional component of the enzyme molecule.

The protein was crystallized from fresh beef liver.[1]

Quantitative emission spectrography and microchemical determination of zinc were performed in duplicate according to methods previously described.[2] Protein weights were determined by trichloroacetic acid precipitation.[2]

Table I presents quantitative spectrochemical and microchemical data on three times crystallized preparations of glutamic dehydrogenase having high specific activity. Preparation 1 contained 333 μg. Zn/g. of protein, preparation 2 contained 322 μg. Zn/g. of protein, and a third preparation contained 260 μg./g. The zinc content of these crystalline preparations was not lowered by prolonged dialysis. The molecular weight of glutamic dehydrogenase has been determined to be about one million.[1] Based on this molecular weight, the number of gram atoms of zinc per mole of enzyme protein vary between 4 and 5 for these preparations.

(1) J. A. Olson and C. B. Anfinsen, *J. Biol. Chem.*, **197**, 67 (1952).
(2) B. L. Vallee and F. L. Hoch, *Proc. Natl. Acad. Sci.*, **41**, 327 (1955).

TABLE I

ANALYSES OF THREE TIMES RECRYSTALLIZED GLUTAMIC DEHYDROGENASE OF BEEF LIVER

Element	Preparation 1 (μg./g. GDH)	Preparation 2 (μg./g. GDH)
Zinc	333	322
Copper	[a]	[b]
Aluminum	63.1	[b]
Barium	27.0	39.9
Calcium	1060	[b]
Magnesium	149	43.0

[a] Not determined. [b] Not detected, and also beryllium, cadmium, cobalt, chromium, iron, lithium, molybdenum, manganese, nickel, potassium, silver, strontium, tin and lead.

Fractionation of beef liver shows an *aggregation of zinc* with purification of the enzyme, and as the activity:protein ratio reaches its maximum value. The metal:protein ratio of all *other elements* studied *decreases* with progressive enzyme purification. The activity:zinc ratio increases progressively with purification, reaching a high value in the third crystals.

The rate of conversion of DPN to DPNH at *p*H 7.7 in the presence of glutamate[1] is inhibited significantly when the enzyme is preincubated with a number of metal binding agents, including sodium sulfide, sodium diethyldithiocarbamate and 1,10-phenanthroline.

These data establish glutamic dehydrogenase as a zinc metalloenzyme according to the criteria published elsewhere.[3]

The oxidation of glutamic acid is the second DPN dependent dehydrogenation reaction known to be catalyzed by a zinc metalloenzyme.[2]

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(3) B. L. Vallee, "Zinc and Metalloenzymes" in "Advances in Protein Chemistry," Vol. X, Academic Press, New York, N. Y., 1955, in press.
(4) Fellow of the National Foundation for Infantile Paralysis.

STEROIDAL SAPOGENINS. XXXI. GENTROGENIN AND CORRELLOGENIN, NEW SAPOGENINS FROM *Dioscorea spiculiflora*[1]

Sir:

Some years ago Marker announced the isolation of botogenin and neobotogenin from *D. mexicana*.[2a,b] The structures assigned had both a 12-keto and a 5,6 double bond. Since substances with these groupings would be particularly desirable as cortisone precursors, there was an extensive but fruitless search made for them. We have now found in several collections of *D. spiculiflora*

(1) Paper XXX: M. E. Wall, submitted in *Experientia*.
(2) (a) R. E. Marker and J. Lopez, THIS JOURNAL, **69**, 2397 (1947); (b) R. E. Marker, *ibid.*, **71**, 2656 (1949).

two isomers which have the structures assigned to botogenin and neobotogenin. Since, however, their melting points and those of their acetates differ greatly from Marker's compounds (Table I) and, since the latter were inadequately characterized, we wish to rename these sapogenins and propose *gentrogenin* (I) and *correllogenin* (II) for them.[3,4] Because of their possible chemical importance, we wish to make a preliminary announcement of their occurrence and structure proof.

TABLE I

	M.p., °C.	
	Sapogenin	Acetate
Botogenin	262	248
Neobotogenin	246–248	234
Gentrogenin	215–216	227
Correllogenin	209–211	213–214

Treatment of the crude sapogenin mixture with Girard's reagent gave a mixture of I and II from which the less soluble I acetate was readily crystallized, (m.p. 227°, $[\alpha]^{25}D$ −56° ($CHCl_3$); calcd. for $C_{29}H_{42}O_5$: C, 74.01; H, 9.00; found: C, 74.10; H, 9.10), which on hydrolysis gave I (m.p. 215–216°, $[\alpha]^{25}D$ −57°; calcd. for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41; found: C, 75.46; H, 9.51). The infrared spectrum of I showed a strong carbonyl peak at 1712 $cm.^{-1}$, a weak band at 836 $cm.^{-1}$ associated with a Δ^5-ethylenic band,[5a,b] and the typical "22a" (= 25D) fingerprint spectrum 980(S), 919(W), 898(S), 863(W) $cm.^{-1}$.[6a,b]

Catalytic reduction of I acetate followed by oxidation with CrO_3–acetic acid gave hecogenin acetate, m.p. 245–247°, infrared spectrum identical to an authentic specimen; Wolff–Kishner reduction of I acetate gave diosgenin, m.p. 204–206°, infrared spectrum identical with an authentic sample. These reactions establish the structure of gentrogenin as 5-20α,22α,25D-spirostene-3β-ol-12-one.[7]

Repeated fractional crystallization of soluble mother liquors gave II acetate, (m.p. 213–214°, $[\alpha]^{25}D$ −60°, calcd. for $C_{29}H_{42}O_5$: C, 74.01; H, 9.00; found: C, 74.10; H, 9.10); infrared spectrum showed two strong bands at 1737, 1713 $cm.^{-1}$ (acetate and 12 carbonyl, respectively), a weak 838 $cm.^{-1}$ band[5a,b] and typical "22b" (= 25 L) bands[6a,b] at 986(S), 920(S), 897(W), and 852(W) $cm.^{-1}$. Catalytic reduction of II acetate followed by mild CrO_3–acetic acid oxidation gave a compound which, from the method of preparation and infrared,[8] we deduce to be 5a-20α,22a,25L-spirostan-3β-ol-12-one 3-acetate, m.p. 214–216°, $[\alpha]^{25}D$ −12°. Infrared shows 1733, 1708 $cm.^{-1}$ carbonyl bands, typical "22b" (= 25L) fingerprint spectrum, and absence of ethylenic 838 $cm.^{-1}$ absorption. Hydrolysis of II acetate gave II, (m.p. 209–211°, $[\alpha]^{25}D$ −69°, calcd. for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41; found: C, 75.14; H, 9.63). Wolff–Kishner reduction of II gave the known yamogenin m.p. 190–194°, infrared essentially identical to an authentic sample. The above reactions establish the structure of correllogenin as 5-20α,22a,25L-spirostene-3β-ol-12-one.

Gentrogenin and correllogenin have been found only in tubers of *Dioscorea spiculiflora*, which occurs in southern Mexico. They are always present in a mixture with diosgenin and yamogenin and constitute 15–25% of the total sapogenin content which averages about 3% on a dry basis.

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(3) In honor of Doctors H. S. Gentry and D. S. Correll, Horticultural Crops Research Branch, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland, who obtained the plant material from which these new sapogenins were isolated.

(4) For precedents for renaming several sapogenins first allegedly isolated by Marker, *cf.* THIS JOURNAL, **75**, 4437 (1953); *J. Chem. Soc.*, 1671 (1955).

(5) (a) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, THIS JOURNAL, **73**, 3215 (1951); (b) C. R. Eddy, M. E. Wall, M. K. Scott, *Anal. Chem.*, **25**, 266 (1953).

(6) (a) M. E. Wall, C. R. Eddy, M. L. McClennan, and M. E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952); (b) R. N. Jones, E. Katzenellenbogen and K. Dobriner, THIS JOURNAL, **75**, 158 (1953).

(7) For present concepts of the stereochemistry of the sapogenin side chain *cf.* I. Scheer, R. B. Kostic and E. Mosettig, THIS JOURNAL, **77**, 641 (1955); J. B. Ziegler, W. E. Rosen and A. C. Shabica, *ibid.*, **77**, 1223 (1955); M. E. Wall, S. Serota and C. R. Eddy, *ibid.*, **77**, 1230 (1955); and M. E. Wall, ref. 1. It seems that natural sapogenins do not differ at C_{22}.

(8) Insufficient material was available for carbon and hydrogen analyses.