

Female Hormone in Bituminous Coal from Shangtung Province

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The study of female hormones becomes more and more intensive, as its clinical applications in the treatment of diseases of women become more and more important. The sources of the female hormone are urine of pregnant and non-pregnant women, urine of pregnant mares, corpus luteum, placenta, etc. But several workers have reported that extracts of various plant products contain the active substance. Walker and Janney reported positive results with extracts of elder leaves and catkins, willow catkins, sprouted oats and rhuba leaves. In a recent publication, Butenandt isolated an active substance from palm leaves. Plants are considered to be the origin of coal, and the latter might, therefore, still contain some active substance from the original plant.

EXPERIMENTAL

The authors analysed eight samples of bituminous coal from different localities in Shangtung province and tested the active substance from each sample. The results are reported below:

Preparation of the Active Extract.—One Kg. of powdered coal was digested with sulfuric acid on a water-bath for six hours. After filtration, the filtrate was extracted with ether. The ether solution after washing with 10% sodium carbonate solution and drying with anhydrous sodium sulfate, was evaporated *in vacuo* and the residue was taken up in 20 cc. of olive oil.

Determination of Potency.—Allen and Doisy (1) have called the amount of potent substance necessary to produce a full change from the negative to the estrual vaginal spread in a castrated rat of approximately 140 Gm. (± 20 Gm.) in weight a "rat unit" (R. U.). The above prepared olive oil solution was divided into several doses, which were injected into the castrated rats. After 48 hours the vaginal smears of the test animals were examined under the microscope to ascertain the estrual condition. The results are tabulated below:

	Sample	R. U./Kg.
(1)	Huang-Shan	100
(2)	Li-Tsia-Ku	80
(3)	He-Shan	200
(4)	Hsin-Tsing	60
(5)	Huang-Tsia-Ta-Wa	50
(6)	Ta-Wa-Tsing	40
(7)	Tao-Hau-Ku	65
(8)	Tsao-Yang-Po	45

SUMMARY

1. The potency of the eight samples from Shangtung Province have been determined.

2. Sample (3) He-Shan possessed the highest potency (200 R. U./Kg.) while

Table I.—Proximate Analysis of Samples of Shangtung Coal

Sample	Moisture, %	Ash, %	Volatile Matter, %	Fixed Carbon, %	Calorific Value (Cal.)
(1) Huang Shan	0.49	9.48	7.93	80.30	8260
(2) Li-Tsia-Mu	0.25	7.78	8.56	83.40	8460
(3) He-Shan	0.41	7.52	16.83	75.24	8060
(4) Hsin-Tsing	0.51	10.24	13.89	75.36	7960
(5) Huang-Tsia-Ta-Wa	0.35	9.35	16.01	74.30	7600
(6) Ta-Wa-Tsing	0.52	13.05	16.49	69.95	7464
(7) Tao-Hua-Ku	0.62	16.21	14.49	68.86	7232
(8) Tsao-Yang-Po	0.74	19.97	15.59	63.82	6410

Table II.—Elementary Analyses of Samples of Shangtung Coal

Sample	C %	H %	O %	N %	S %
(1) Huang-Shan	80.63	1.90	17.47	0.46	2.48
(2) Li-Tsia-Ku	83.24	1.89	14.87	1.24	3.21
(3) He-Shan	80.83	2.09	17.08	1.28	0.64
(4) Hsin-Tsing	78.60	2.05	19.36	1.25	3.69
(5) Huang-Tsia-Ta-Wa	78.93	2.14	18.93	1.38	2.11
(6) Ta-Wa-Tsing	76.00	2.23	21.77	1.35	0.79
(7) Tao-Hua-Ku	73.69	1.82	24.40	1.27	0.75
(8) Tsao-Yang-Po	68.72	2.06	29.22	1.25	0.83

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sample (6) Ta-Wa-Tsing possessed the lowest (40 R. U./Kg.)

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Pectin Studies, V. Organic Base Derivatives of Pectinic and Pectic Acids

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The present conception of pectin as a chain molecule made up largely, if not entirely, of galacturonic acid anhydride units, part of which are methylated, greatly simplifies an interpretation of pectin properties (2), (6), (8), (9). Acid properties are conferred by the free carboxyl groups resulting from partial demethoxylation. The reactions of pure pectin correctly called pectinic acid are, therefore, essentially those of a typical organic acid of high molecular weight (1). The colloidal properties presumably are indicative of the number of units in the chain, and the only difference between pectic acid and pectinic acid is that the former is more completely demethoxylated than the latter. Both pectins and pectic acid of greatly varying molecular size, as indicated by specific viscosity and gel properties, do exist; but usually pectic acid will be found to be of considerably lower molecular weight as indicated by specific viscosity, than is normal for pectin. This is a normal result of conditions which cause demethoxylation. However, by slow, strong acid, low temperature demethoxylation one can avoid the splitting of the long chains (7). In this manner pectic acid of high molecular weight can be prepared,

while by the usual alkaline method of demethoxylation low molecular weight pectic acid usually is obtained.

The reaction of pectin and pectic acid with certain metal ions to form insoluble compounds has long been known. Such insoluble precipitates have been described by a number of writers from Braconnot to von Fellenberg. From a study of several pectins differing in methoxyl content, von Fellenberg correctly concluded that "the more methoxyl groups split off the more acid groups present in the pectin molecule and the easier does coagulation with metal salts occur" (5).

Nevertheless, even more recently, some have failed to recognize the essentially stoichiometric relationships which control reactions that occur between pectin and either inorganic or organic bases. Some commercial pectin preparations may be but slightly acid in reaction due to a neutralization of the free acid groups. However, the combined cations are readily removed by washing with acidified alcohol and the excess acid can be removed by washing with dilute alcohol. Such pectin in 1% solution will have a p_H well below 3.0, and should preferably be referred to as pectinic acid. It is questionable whether a "neutral" pectin, that is a completely methylated pectin, exists in the free state. Most pectin samples observed by the authors have equivalent weights of not over 1200, and some commercial preparations run as low as 400 equivalent weight while pectic acid has an equivalent weight of about 205. It is not logical to assume a difference in type of reactions entered into by pectic acid and such pectinic acids. The difference is rather one of degree due to the difference in equivalent weight. Naturally one may expect pectic acid to form insoluble precipitates with many salts which do not precipitate pectins of high equivalent weight because the latter do not combine with enough of the precipitating ion to become insoluble. For example, pectin of high combining weight reacts with calcium to form calcium pectinate although it does not form a precipitate with calcium, whereas insoluble calcium pectate provides the well-known method for determining pectic acid.

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