Chemical Studies on Oil Bearing Seeds. II. Hubam Clover Seed

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HUBAM sweet clover is an annual variety of white sweet clover and is known botanically as *Melilotus alba annua* (9). It was first observed in Mississippi as early as 1898 and was later (1916) isolated from a planting of biennial white sweet clover and brought to general attention by H. D. Hughes of the Iowa Agricultural Experiment Station (2, 4, 6). It is believed to have originated in Alabama (4). The name Hubam is derived from the names Hughes and Alabama.

In recent years Hubam clover has attained considerable recognition as a forage crop and as a soil builder. It has been used very satisfactorily as a cover crop and as a green manure crop. By crop rotation with cotton and other grains Hubam clover is said in some cases to produce as much as 50% increase in yields because of the nitrogen enrichment of the soil (8). This is a very strong factor in favor of Hubam clover as a southern legume crop. In the South, and particularly in Texas and other areas of the Southwest, it is grown for seed production and is assuming importance as one of the lucrative cash crops.

From the standpoint of agricultural economics it is considered especially desirable that a crop serve as a soil builder and at the same time provide an attractive cash income without being unduly difficult to cultivate and harvest. Hubam clover satisfies all of these prerequisites. Yields of 700 pounds of seed per acre are not unusual, and the average price to the farmer has made it an attractive cash crop as well as a soil builder. Seeding requires about 20 pounds to the acre, no cultivation is necessary, and harvesting is done by combine. Losses during harvest are minimized and 85% of the seed is saved by cutting and windrowing in a single operation, followed by threshing out of the windrow with a combine (8).

During the past few years there has been a remarkable increase in acreage planted to Hubam clover. In 1947, for the first time, seed production exceeded the market demand, and producers were left with a surplus of this commodity. In anticipation of this trend, and in view of the tremendous importance of Hubam clover to southern agriculture, extensive chemical investigations have been carried out in the laboratories of the Texas Research Foundation to determine the chemical composition of Hubam clover seed and to find industrial applications for its principal components. The object of this paper is to report the results of our investigations to date and to give a preliminary evaluation of Hubam clover as a potential source of industrial raw materials.

Seed Analysis. Mature samples of Hubam clover seed, freed from all foreign matter, were ground to pass a 40-mesh screen and the ground material was subjected to a series of analyses, the results of which are reported in Table I. The methods were those accepted as official procedures (1, 3).

Approximately 7% of the composition of the seeds remains unknown. The smallness of the seed and the intimacy with which the constituents are in contact with one another makes a quantitative determination of each very difficult. It is quite likely that the seeds contain more carbohydrate material than is indicated in the above table.

TABLE I

Composition of Hubam Clover Seeds

	Moisture-Free Basis
	%
Oil (petroleum-ether soluble)	7.6
Crude protein (N x 6.25)	39.4
Starch	3.0
Pentosans	8.1
Total sugars (as glucose)	2.7
Crude fiber	12.9
Ash	4.1
Coumarin, resins, glucosides, pigments, etc., approx. (Obtained by difference between alcohol-soluble and petroleum-ether soluble extracts)	15.0
Total	92.8

It is observed that the seeds are relatively low in oil content, high in percentage protein, and low in carbohydrate materials. The percentage protein compares favorably with that of the soybean and cottonseed. In addition, the seeds contain notable amounts of minor constituents. These include two unidentified pigments. One is red and the other is yellow in acidic solutions while both are quite colorless in basic solutions. The seeds also contain a small amount of resinous matter, some glucosides, and about 2% of coumarin.

After some earlier observations of this laboratory in which it was noted that acetone extracted approximately twice as much material from Hubam clover seed as petroleum ether, it was decided to carry out a series of quantitative extractions on the ground seeds using solvents selected from the various classes of organic compounds in order to determine the relative yields obtainable. The results are shown in Table II. Cold extractions were made by means of the Soxhlet extraction apparatus.

TABLE II

Yields Obtained by Cold Extraction of Hubam Clover Seed With Various Solvents

Solvent Used	Extract on a Moisture-Free Basis
	%
Petroleum-ether (b.p. 35-65° C.)	7.32
Toluene	8.34
Benzene	8.42
Ethyl ether	9.01
Chloroethane	9.45
Ethyl acetate	12.05
Acetone	13.30
Dioxane	18.22
Ethanol*	22.11

*Added as anhydrous ethanol to ground seeds containing 8 to 12% moisture.

It is of interest to note that the smallest yield was obtained with petroleum ether as the solvent. Although this solvent effects a complete removal of the glyceride oils with a minimum of other constituents, the high yield of extract (22.1%) obtained with anhydrous ethanol attracts interest as a satisfactory means of removing a number of useful materials from the seed in one operation. Further studies are now in progress to determine the constituents present in the alcoholic extract and to develop methods for their separation and purification.

Seed Proteins. Hubam clover meal, which had been previously extracted with petroleum ether, was ground to pass a 100-mesh sieve and then subjected to the usual treatments necessary to obtain the various classes of proteins on the basis of differences in solubility. The results are shown in Table III.

TABLE III
Composition of Hubam Clover Seed Proteins

	Moisture-Free Basis	Protein (N x 6.25)
	%	%
Globulins	22.4	107.0
Glutelins	6.0	78.9
Albumins	2.5	23.8
Prolamines	0.6	......
Total	31.5	
Crude protein (See Table I)	39.4	

It appears from Kjeldahl nitrogen determinations on the protein fractions that the albumin fraction is largely non-protein in character. The glutelin and globulin fractions may be different percentage protein than is indicated in column two because of the arbitrary nature of the factor 6.25, which is based on the assumption that all proteins contain 16% nitrogen. This, however, is not true of isolated protein fractions which are known to differ in nitrogen percentage.

The above data do not indicate any outstanding peculiarities in the protein composition of Hubam clover seed. Further investigations on the amino acid composition of the above mentioned protein fractions will be necessary to show details of composition. Practically no information dealing with the protein composition of white clover seed can be found in the literature.

Seed Oil. Hubam clover seed oil was obtained from the ground seeds by successive batch extractions, using petroleum ether as the solvent. The solvent was then removed from the extracted oil by distillation and reduced pressure in order to minimize injury to the oil by heat. The crude oil thus obtained was yellowish brown in color and possessed an odor characterized by dissolved traces of coumarin. Table IV contains a summary of physical and chemical data on this oil.

TABLE IV
Some Physical and Chemical Characteristics of Hubam Clover Seed Oil

Property	
Refractive inedx, 25°	1.4741
Specific gravity, 25/25°	0.9185
Melting point	—23° C.
Solidification point	—25° C.
Saponification value	180.1
Iodine value (Wijs)	141.0
Unsaponifiable matter (per cent)	5.9
Free fatty acids (per cent)	0.3
Saturated fatty acids (per cent)	1.5
Reichert meissl value	1.4
Polenske value	0.2
Acetyl value (Andre-Cook)	21.1

The oil obtained by alcohol extraction is very dark in color, being heavily laden with pigments and some very odorous substances. In strong concentration the odor is unpleasant but in greater dilution is somewhat pleasant and similar to tobacco. On evaporation of the solvent, a black solid, amounting to about 50% by weight of the extract, separates out leaving an almost black liquid oily fraction.

The relatively high iodine value (141.0) places Hubam clover seed oil in the lower range of the drying oils according to this method of classification. However, thin layers of the oil on glass harden uniformly to a tough, non-tacky film within a few days without the aid of driers and in much less time than would be predicted on the basis of the iodine value.

The relatively high percentage of unsaponifiable matter (5.9%) indicates that Hubam clover seed oil may be a good commercial source of sterols and phosphatides. These constituents are being investigated further.

A preliminary study of the fatty acid composition of Hubam clover seed oil indicates that there is only about 2% of saturated acids present. The unsaturated acids consist largely of oleic and linoleic acids, containing approximately 50% of the latter. The relatively low saponification value suggests the presence of significant quantities of a 20-carbon, or possibly 22-carbon, acid with one double bond. Further studies on the fatty acid composition of this oil are now in progress.

Coumarin. The properties of coumarin extracted from Hubam clover seed and purified by recrystallization were compared with those of synthetic (Eastman Kodak) coumarin and were found to agree in solubility, melting point (separately and when mixed), sublimation point, specific needle formation on mercuric chloride in the presence of congo red, and in ultraviolet absorption spectrum (see Fig. 1), using the Beckman D. U. Spectrophotometer.

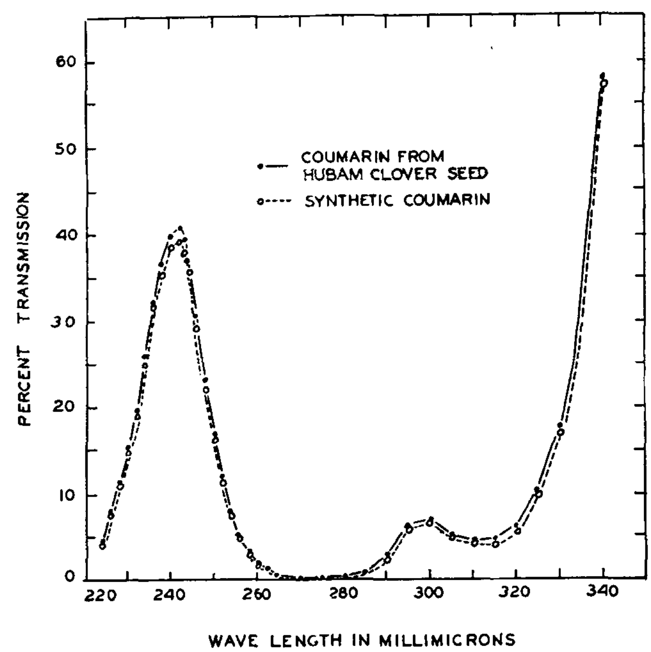

FIG. 1. Ultraviolet absorption spectrum of coumarin.

Discussion and Summary

On the basis of information gathered during our investigations up to the present time it is felt that Hubam clover holds promise as a source of the following valuable industrial raw materials:

1. An oil which has very satisfactory drying properties and should be useful for the manufacture of paints, varnishes, linoleum, and oilcloth, and other types of protective and decorative coatings. The oil represents 7.6% of the dry weight of the seed. While

production of the oil would be on a relatively small scale because of the low oil content of the seed, nevertheless it should prove to be a valuable minor source of drying oil and worthy of some attention during the current shortage of drying oils.

2. Coumarin, comparable in quality to the synthetic product, representing 2% of the dry seed weight. It is useful in the manufacture of artificial vanilla flavoring, candy, pastries, baked goods, soap, tobacco, cosmetics, and perfumes.

3. A protein-rich meal which may be useful in the manufacture of adhesives, fibers, plactics, protective coatings, etc., and as a source of special amino acids. After being subjected to hot alcohol extraction (to remove the oils, glucosides, and other bitter principles, the sterols, phosphatides, resins, pigments, and coumarin) Hubam clover meal is assumed to be free of possible toxic compounds. Coumarin, which under certain circumstances gives rise to dicoumarol, the causative agent for the hemorrhagic sweet clover disease, is thus removed. The resulting product should be useful as a high protein supplement in foods and feeds. This meal averages about 47% protein. The alcoholic extract may be further processed for its useful ingredients including coumarin pigments (useful as indicators, antioxidants, medicinals, etc.), sterols (useful in the preparation of pharmaceuticals), phosphatides (useful in emulsifying agents, cosmetics, pharmaceuticals, etc.), and other constituents as yet unidentified.

Although Hubam clover has not been found to be especially rich in any one material, it is felt that the combined value of the various constituents present in the seed should ultimately make it an attractive source of basic raw materials.

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A Spectrophotometric Method for the Determination of Color of Glyceride Oils

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Introduction

NUMEROUS proposals have been made for methods to avoid the difficulties attendant upon visual methods of color measurement in the examination of animal and vegetable fats and oils. The most recent developments have been spectrophotometric methods, three of which are mentioned by Thomson (1). In two of these instances the spectrophotometric readings were correlated with Lovibond values and in the third with FAC standards. Such systems represent a distinct advance, but it is felt that expression of the photometric values in terms of a less arbitrary standard would be a more desirable procedure. Not only are Lovibond glasses difficult to obtain and frequently somewhat inaccurate, but they consist of an arbitrary series of filters whose transmissions do not correspond to the primary color sensitivities of the eye. Results obtained with the Lovibond system are appreciably dependent upon the color vision and judgment of the observer. If the procedure recommended by the A.O.C.S. (2) be followed, the Lovibond glasses used are often not those which give the best color match.

Due to varying quantities of colored substances in oils it is not possible to select any single wave length at which the transmittance will be an exact function of the color, total transmission, or the visual transmission of the oil. Inasmuch as color, rather than absorption at any specific wave length, is the value it is desired to measure, expression of the results in terms of tri-stimulus values would be the only entirely satisfactory method. If, however, it is necessary or desirable to express the color in terms of a single value, the Y of the tri-stimulus values may be used as it is a direct measure of the transparency in terms of visibility function (3).

Due to the general shape of the spectral transmission curves of glyceride oils it seemed probable that the tri-stimulus values could be closely approximated by functions of the transmission values at three properly selected wave-lengths. This is essentially the determination of the tri-stimulus values by the method of weighted ordinates but involves a great reduction in the number of ordinates.

Apparatus

The data presented here are derived from transmission curves drawn by a General Electric recording spectrophotometer having an effective slit width of 10 millimicrons and using absorption cells of 10.0 and 50.0 mm. sample lengths. Any instrument of corresponding slit width and accommodating cells up to 50 millimeters may be used. The Beckman quartz spectrophotometer, which is widely used in the oil industry, is satisfactory if equipped with the interchangeable cell compartments. If instruments with cell lengths of less than 50 millimeters be used, the transmissions may, when necessary, readily be calculated to a 50-millimeter basis with satisfactory results by the use of Bouguer's (or Lambert's) Law. This law may be stated as $T = t^x$, where t is the transmission factor of a material of unit thickness, and T the transmission factor of the material of x thickness.