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No. 107

18.

THE CONSTITUENTS OF THE FLOWERS OF TRIFOLIUM INCARNATUM

BY

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LXXIX.—*The Constituents of the Flowers of Trifolium incarnatum.*

By Harold Rogerson.

The flowers of *Trifolium incarnatum*, Linné—the so-called "Carnation or Crimson Clover"—appear never to have been chemically examined. Inasmuch as the flowers of the common red clover (*Trifolium pratense*, Linné) have recently been made the subject of a thorough investigation (Power and Salway, this vol., p. 231), it was deemed desirable also to examine those of the less widely cultivated "carnation clover," especially as the latter possess such very distinctive characters. It has thus been ascertained that the flowers of the two above-mentioned species of *Trifolium* exhibit appreciable differences with respect to their constituents, and a complete summary of the results of the present investigation is given at the end of this paper.

EXPERIMENTAL.

The material employed in this investigation consisted of the flowering tops of *Trifolium incarnatum*, which had been collected during the month of June from a field of the cultivated plant in Kent.

A portion (10 grams) of the dried material was first tested for the presence of an alkaloid, but the reactions obtained were very slight, and indicated the presence of not more than traces of such a substance.

Another portion (25 grams) of the dried material was successively extracted in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 100°, were obtained:

Petroleum (b. p. 35—50°)	extracted	0·50	gram	= 2·00	per cent.
Ether	,,	0·32	,,	= 1·28	,,
Chloroform	,,	0·20	,,	= 0·80	,,
Ethyl acetate	,,	1·03	grams	= 4·12	,,
Alcohol	,,	4·60	,,	=18·40	,,
Water	,,	1·29	,,	= 5·60	,,
	Total...	7·94	grams	=31·76	per cent.

For the purpose of a complete examination, 94·35 kilograms of the flowers were collected. This material, after careful drying, amounted to 20·75 kilograms, or 22 per cent. of the original weight. The entire amount of this material was ground, and then extracted by continuous percolation with hot alcohol. After the removal of the greater portion of the alcohol, a dark-coloured extract was obtained, which amounted to 6·35 kilograms.

Distillation of the Extract with Steam: Separation of an Essential Oil.

The whole of the above-mentioned extract was mixed with water, and steam passed through the mixture for several hours. The distillate (12 litres) contained some drops of oil floating on the surface. It was thoroughly extracted with ether, the ethereal liquid being dried, and the solvent removed, when 6 grams of an essential oil were obtained. The yield of the latter was equivalent to 0·029 per cent. of the dried, or 0·006 per cent. of the fresh flowers.

This essential oil, when distilled under diminished pressure, passed over between 120° and 180°/15 mm. It was a pale yellow, mobile liquid, which darkened slightly on keeping, and possessed a strong, persistent odour. It gave a brown colour with ferric chloride, and a strong furfuraldehyde reaction. Its constants were as follows: d $20°/20° = 0·9597$; α_D $-1°48'$ in a 1-dcm. tube.

After the distillation of the extract with steam, as above described, there remained a dark-coloured aqueous liquid (A), together with a quantity of green, resinous material (B). These products were separated by filtration when cold, and the resin was washed repeatedly with hot water until nothing further was removed, the washings being added to the aqueous liquid.

Examination of the Aqueous Liquid (A).

Isolation of Benzoic and Salicylic Acids.

The aqueous liquid was repeatedly extracted with ether, the combined ethereal liquids concentrated to the volume of 1 litre, and then shaken with successive portions of aqueous ammonium carbonate. The alkaline liquids were united, acidified, and again extracted with ether, the ethereal liquid being washed, dried, and the solvent removed. A little resinous material was thus obtained, from which, by repeated treatment with light petroleum, a small amount of a crystalline product was extracted. The latter was recrystallised from water, when it separated in plates and needles, thus indicating it to be a mixture. For the purpose of effecting a separation of the acids, they were converted into their ethyl esters, the ethereal solution of which was first shaken with aqueous sodium carbonate to remove any unchanged acid, and subsequently with an aqueous solution of sodium hydroxide. On evaporating the ether, a small amount of an oily liquid was obtained, which possessed the odour of ethyl benzoate, and, on hydrolysis, yielded an acid which, when crystallised from water, separated in plates, melting at 121—122°. This substance was evidently benzoic acid, and its identity was confirmed by mixing it with a little of the latter acid, when the melting point remained unchanged.

The alkaline liquid obtained by shaking the ethereal solution of the above-mentioned esters with aqueous sodium hydroxide was boiled for some time and acidified, when a small amount of substance was obtained which, when crystallised from water, separated in needles, melting at 154—156°. This substance gave a violet coloration with ferric chloride, and evidently consisted of salicylic acid, since its melting point remained unchanged when mixed with this acid.

The resinous material from which the benzoic and salicylic acids had been separated was further treated with a mixture of light petroleum and ether. On removing the solvent a very small amount of a crystalline substance was obtained, which, when recrystallised from water, separated in needles, melting and decomposing at 206—207°. This substance appeared to be *p*-coumaric acid, which, together with salicylic acid, has recently been shown to be a constituent of red clover flowers (*loc. cit.*). None of the above-mentioned acids was obtained in an amount sufficient for analysis.

Isolation of Quercetin, $C_{15}H_{10}O_7$.

The ethereal extract of the aqueous liquid, after being treated with ammonium carbonate as above described, was shaken with twelve successive portions of aqueous sodium carbonate. These alkaline liquids were separately acidified, and the resulting yellow precipitates collected, washed, and dried. They were then united to form five fractions, which were acetylated. The acetyl derivatives of the first four fractions, having been found to be identical, were united, and hydrolysed by heating with dilute sulphuric acid in aqueous alcohol. On subsequently removing the alcohol by distillation in a current of steam, a small amount (0·4 gram) of a yellow substance separated in small, yellow needles, which melted and decomposed at 305°. After drying at 135—140°, the substance was analysed:

0·1022 gave 0·2242 CO_2 and 0·0366 H_2O. C=59·8; H=3·9.

$C_{15}H_{10}O_7$ requires C=59·6; H=3·3 per cent.

A portion of the substance was again converted into its acetyl derivative by heating with acetic anhydride. On adding about an equal volume of light petroleum (b. p. 90—120°) to the hot solution, a crystalline product almost immediately separated, which melted at 195—196°. This substance, after drying at 110°, was analysed:

0·0920 gave 0·1962 CO_2 and 0·0350 H_2O. C=58·2; H=4·2.

$C_{15}H_5O_7(CO{\cdot}CH_3)_5$ requires C=58·6; H=3·9 per cent.

From these results it is evident that the above-described yellow colouring matter was quercetin. It has previously been shown that this compound is contained in two other species of clover in the form of a glucoside, having been obtained by Perkin and Phipps (Trans., 1904, **85**, 58) from white clover flowers (*Trifolium repens*), and quite recently by Power and Salway (*loc. cit.*, p. 244) from the flowers of the common red clover (*Trifolium pratense*).

Isolation of Pratol, $C_{16}H_{12}O_4$.

The final fraction of the above-mentioned sodium carbonate extracts of the ethereal liquid yielded, when acidified, a precipitate which could not be directly purified by crystallisation. It was therefore acetylated, and the acetyl derivative so obtained crystallised from a mixture of alcohol and ethyl acetate, when it separated in needles, melting at 166°. The amount of this substance was insufficient for analysis, but on comparing it with the acetyl derivative of pratol, $C_{16}H_{12}O_4$ (Power and Salway, *loc. cit.*, p. 233), the two compounds were found to be identical.

Isolation of a New Glucoside, Incarnatrin, $C_{21}H_{20}O_{12},3H_2O$.

The original aqueous liquid (A), which had been repeatedly extracted with ether as above described, was shaken with many successive portions of amyl alcohol. These liquids were united, washed with water, and concentrated under diminished pressure to a volume of about 500 c.c., when, after some time, a yellowish-brown precipitate was deposited, which was collected and washed with light petroleum. By the further concentration of the amyl-alcoholic liquid, a much larger quantity of a yellow precipitate was obtained, which was similarly collected and washed. The final amyl-alcoholic filtrate was concentrated and treated with light petroleum, when a further small quantity of yellow product was obtained. Each of these precipitates was dissolved in a small amount of alcohol, and the solutions kept for some time, when small amounts of a crystalline product were obtained, which were finally united and crystallised from water. A quantity (0·4 gram) of a substance then separated in faintly yellow, prismatic needles, which softened at 165°, and decomposed at 242—245°. The substance was first dried in a vacuum, and then analysed:

0·1490 lost 0·0136 H_2O at 115—120°. $H_2O = 9·1$.
0·1354 * gave 0·2702 CO_2 and 0·0600 H_2O. $C = 54·4$; $H = 4·9$.
$C_{21}H_{20}O_{12},3H_2O$ requires $H_2O = 10·4$ per cent.
$C_{21}H_{20}O_{12}$ requires $C = 54·3$; $H = 4·3$ „

From these results it would appear that this substance possesses the formula $C_{21}H_{20}O_{12},3H_2O$, but that a little of the water of crystallisation had been lost on drying in a vacuum.

The glucosidic character of this substance was established by heating a portion of it for about two hours with 2·5 per cent. aqueous sulphuric acid. On cooling, a yellow, crystalline substance was deposited, which yielded an acetyl derivative melting at

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* Anhydrous substance.

195—196°. The aqueous acid liquid was treated with baryta for the removal of the sulphuric acid, filtered, and concentrated. It then yielded an osazone, which, after recrystallisation from dilute pyridine, melted at 203—205°.

It is evident from the above results that the substance under examination was a glucoside of quercetin, and that its hydrolysis can be represented by the following equation:

$$C_{21}H_{20}O_{12} + H_2O = C_{15}H_{10}O_7 + C_6H_{12}O_6.$$

Hitherto, only two other glucosides appear to have been isolated which give quercetin and glucose on hydrolysis, namely, osyritrin, $C_{27}H_{28}O_{16},3H_2O$ (m. p. 185°), which was first obtained by A. G. Perkin from Cape sumach (Trans., 1897, **71**, 1131), and quercimeritrin, $C_{21}H_{20}O_{12},3H_2O$ (m. p. 247—249°), which has quite recently been isolated by the same investigator from cotton flowers (Trans., 1909, **95**, 2181). As there was a possibility of the above-described glucoside being identical with quercimeritrin, a specimen of the latter was kindly supplied by Professor A. G. Perkin for the purpose of comparison, but they were found to differ in their character. The glucoside obtained from *Trifolium incarnatum* is therefore a new compound, and it is proposed to designate it *incarnatrin*, with reference to the specific name of the plant.

Incarnatrin is hydrolysed by emulsin, and is therefore a β-glucoside. It dissolves slowly in concentrated sulphuric acid with a yellow colour, and the solution soon develops a green fluorescence.

The original aqueous liquid, after being freed from amyl alcohol by means of steam, was treated with an excess of a solution of basic lead acetate. A bulky, yellowish-brown precipitate was thus obtained, which was collected, well washed with water, then suspended in water, decomposed by hydrogen sulphide, and the mixture filtered. The resulting liquid, when concentrated, had a dark red colour, and appeared to contain only a small amount of tannic matter.

The filtrate from the basic lead acetate precipitate was treated with hydrogen sulphide in order to remove lead, and concentrated under diminished pressure. A syrupy liquid was thus obtained which evidently contained a quantity of sugar, for it readily reduced Fehling's solution, and yielded *d*-phenylglucosazone, melting at 205—206°.

Examination of the Resin (B).

This resin, as already indicated, represented that portion of the original alcoholic extract which was insoluble in water. At the ordinary temperature, it was a soft, green mass, and amounted to

856 grams. It was treated with hot alcohol, when a portion (88 grams) remained undissolved in the form of a hard, brown solid.

For the examination of the soluble portion of the resin, a quantity (192 grams) representing 1·59 kilograms of the original alcoholic extract was employed. This was mixed with purified sawdust, the thoroughly dried mixture being then successively extracted in a Soxhlet apparatus with petroleum (b. p. 35—50°), ether chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resin.

This extract was a soft, dark green mass, amounting to 157 grams. It was dissolved in ether, and the ethereal liquid extracted with a solution of ammonium carbonate, which, however, removed nothing. On subsequently shaking the ethereal liquid with successive portions of aqueous potassium carbonate, a quantity of material was removed. The alkaline liquids thus obtained were therefore acidified and extracted with ether, when a small amount of substance remained undissolved. This substance, which was very sparingly soluble in ether, was collected, dissolved in dilute alcohol, and the solution treated with animal charcoal. On subsequently concentrating the solution, the substance separated in glistening laminæ, which melted and decomposed at 235°, but the amount was not sufficient for its further examination.

The ethereal liquid, from which the above-mentioned substance had been removed by filtration, was washed, dried, and the solvent removed. A quantity of fatty acids was thus obtained, together with some resinous matter. In order to purify the acids, they were converted into their ethyl esters, and the latter distilled under diminished pressure. The esters were then hydrolysed, and the regenerated acids distilled under diminished pressure, when they passed over between 220° and 280°/15 mm. as a viscid liquid, which solidified on cooling to a white, crystalline mass. The acids thus obtained amounted to 16·5 grams, and they were examined in connexion with a similar product obtained from the petroleum extract after its hydrolysis.

The ethereal liquid which had been extracted with potassium carbonate, as above described, was subsequently shaken with a solution of sodium hydroxide, which, however, removed only a little resinous material.

Hydrolysis of the Petroleum Extract.

After extracting the ethereal solution of the petroleum extract with alkalis, as above described, the ether was evaporated, when a quantity (90 grams) of a dark green, resinous mass was obtained. This was hydrolysed by heating with an alcoholic solution of potassium hydroxide, after which water was added, the alcohol removed, and the alkaline solution of potassium salts extracted with ether. The ethereal liquid was then washed, dried, and the solvent evaporated, when 30 grams of a dark yellow solid were obtained.

Isolation of Incarnatyl Alcohol, $C_{34}H_{69}{\cdot}OH$, *and Hentriacontane,* $C_{31}H_{64}$.

The above-mentioned solid, representing the unsaponifiable constituents of the petroleum extract, was dissolved in 1 litre of hot alcohol, and the solution allowed to cool, when a quantity (8 grams) of a sparingly soluble substance separated. This was collected, washed with a little alcohol, and dried, when it was found to consist chiefly of a hydrocarbon with a small amount of an oxygenated compound, which presumably was an alcohol. In order to effect their separation, 5 grams of the mixture were heated with an equal weight of phthalic anhydride for about two hours at 140°, the product being subsequently dissolved in a mixture of chloroform and ether, and the solution shaken with aqueous sodium carbonate. A solid compound then separated, which evidently was the sodium salt of an acid phthalic ester. This was hydrolysed with an alcoholic solution of potassium hydroxide, when, on cooling, a substance was deposited which, after recrystallisation from a mixture of ethyl acetate and alcohol, separated in needles, melting at 72—74°:

0·0784 gave 0·2370 CO_2 and 0·1024 H_2O. C=82·4; H=14·5.

$C_{34}H_{70}O$ requires C=82·6; H=14·2 per cent.

In order further to characterise this substance, a portion of it was heated with benzoyl chloride, the excess of the latter having been removed by heating the mixture with a little absolute alcohol and subsequent distillation with steam. The precipitated benzoyl derivative was then crystallised from alcohol, when it separated in small needles, melting at 58—60°.

From the above results it is evident that the substance $C_{34}H_{69}{\cdot}OH$ is identical with an alcohol previously obtained from the wax of the humble-bee by Sundwik (*Zeitsch. physiol. Chem.*, 1898, **26**, 58), who recorded its melting point as 74—75°, and that of its benzoyl derivative as 55°. As no name was assigned by Sundwik to this compound, it is proposed to designate it incarnatyl alcohol, with reference to the species of clover, *Trifolium incarnatum*, from which it has now been isolated.

The chloroform-ether liquid from which the *sodium incarnatyl phthalate* had separated was dried, and the solvent evaporated.

The residue thus obtained was heated with an alcoholic solution of potassium hydroxide in order to remove unchanged phthalic anhydride, when, on cooling, a small amount of a substance was deposited. This was collected, dried, and crystallised from ethyl acetate, when it separated in pearly leaflets, melting at 68°, and was identified as hentriacontane. (Found, C=84·9; H=14·9. Calc., C=85·3; H=14·7 per cent.)

Isolation of a Phytosterol, $C_{27}H_{46}O$.

The alcoholic liquid from which the above-described mixture of incarnatyl alcohol and hentriacontane had separated was concentrated to about half its volume, when a further quantity of solid was deposited. This was collected, and found to consist of a mixture of hentriacontane and a phytosterol. On concentrating the mother liquors, an additional quantity of substance was obtained, which, after crystallisation from a mixture of ethyl acetate and dilute alcohol, separated in fine, glistening needles, melting at 135—136°:

0·4994 lost 0·0248 H_2O at 110°. $H_2O=4·9$.

0·1056 * gave 0·3244 CO_2 and 0·1160 H_2O. C=83·8; H=12·2.

$C_{27}H_{46}O,H_2O$ requires $H_2O=4·5$ per cent.

$C_{27}H_{46}O$ requires C=83·9; H=11·9 per cent.

This substance is thus seen to agree in composition with a phytosterol, and it yielded the colour reaction of this class of compounds. A determination of its optical rotatory power gave the following result:

0·2478 of anhydrous substance, made up to 20 c.c. with chloroform, gave α_D $-1°2'$ in a 2-dcm. tube, whence $[\alpha]_D$ $-41·7°$.

The acetyl derivative, when crystallised from acetic anhydride, separated in needles, melting at 124—125°.

0·1860, made up to 20 c.c. with chloroform, gave α_D $-0°49'$ in a 2-dcm. tube, whence $[\alpha]_D$ $-43·9°$.

The final mother liquors, after the separation of the above-described phytosterol, were evaporated until all the alcohol was removed. The residue consisted of a semi-solid mass, which was distilled under diminished pressure, and then amounted to 9·4 grams. It was found to consist chiefly of liquid hydrocarbons, with some oxygenated substances, but from it there could be isolated only a very small quantity of an alcohol, which crystallised in leaflets, melting at 88°.

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* Anhydrous substance.

Identification of the Fatty Acids.

The alkaline, aqueous solution of potassium salts, from which the substances described above had been removed by extraction with ether, was acidified and again extracted with ether, the ethereal solution being washed, dried, and the solvent removed. A quantity (9·3 grams) of fatty acids was thus obtained, which, when distilled under diminished pressure, passed over between 220° and 280°/15 mm. As these acids distilled within the same range of temperature as those previously obtained, which existed in the flowers in the free state, for the purpose of their examination the two portions were mixed.

Twenty-five grams of the total mixed acids were converted into their lead salts, and the latter digested with ether, when a portion was dissolved. Both the soluble and insoluble portions were decomposed by hydrochloric acid, and the regenerated fatty acids purified by distillation under diminished pressure. The soluble portion of the lead salts yielded 12·6 grams of liquid acids, whilst the insoluble portion gave 10·5 grams of solid acids.

The Liquid Acids.—These acids, when distilled under diminished pressure, passed over between 230° and 250°/15 mm. A determination of the iodine value gave the following result:

0·2358 absorbed 0·3745 iodine. Iodine value = 158·8.

This indicated that the liquid acids consisted of a mixture, containing some acid or acids of a higher degree of unsaturation than oleic acid.

In order to obtain more definite information respecting the composition of the above mixture, a quantity (7·5 grams) of it was oxidised with potassium permanganate according to the method described by Lewkowitsch (*Chemical Technology and Analysis of Oils, Fats, and Waxes*, 1904, Vol. I, p. 360). The chief product of the oxidation was an acid melting at 156—158°, which analysis showed to be tetrahydroxystearic (sativic) acid (Found, C = 61·7; H = 10·5. Calc., C = 62·1; H = 10·3 per cent.). In addition to this acid, small amounts of dihydroxystearic acid (m. p. 125—127°) and hexahydroxystearic acid (m. p. 168—170°) were obtained.

The above results would therefore indicate that the unsaturated acids consisted chiefly of linolic acid, with smaller amounts of oleic and *iso*linolenic acids.

The Solid Acids.—These acids were fractionally crystallised from alcohol, when two fractions were obtained, which melted respectively at 50—52° and 55—57°.

Fraction 50—52°.—This fraction was analysed, with the following result:

0·1360 gave 0·3752 CO_2 and 0·1588 H_2O. C=75·2; H=12·9.

$C_{16}H_{32}O_2$ requires C=75·0; H=12·5 per cent.

$C_{18}H_{36}O_2$ „ C=76·1; H=12·7 „

The above fraction thus appeared to consist of a mixture of palmitic and stearic acids.

Fraction 55—57°.—On recrystallising this fraction from glacial acetic acid, it yielded palmitic acid (m. p. 60—61°: Found, C=74·8; H=12·8. Calc., C=75·0; H=12·5 per cent.).

Ethereal Extract of the Resin.

Isolation of Trifolianol, $C_{21}H_{34}O_2(OH)_2$.

During the extraction of the resin with ether it was observed that a sparingly soluble substance had separated. This was collected, washed, and dried, when it amounted to 2·9 grams. As it contained some green colouring matter, it was first treated with alcohol, and subsequently recrystallised several times from dilute pyridine, when it was obtained in minute, colourless crystals, melting and decomposing at 295—300°. It yielded colour reactions similar to those given by the phytosterols:

0·1014 gave 0·2662 CO_2 and 0·0948 H_2O. C=71·6; H=10·4.

$C_{21}H_{36}O_4$ requires C=71·6; H=10·2 per cent.

A determination of its optical rotatory power gave the following result:

0·2944, made up to 20 c.c. with pyridine, gave α_D $-1°18'$ in a 2-dcm. tube, whence $[\alpha]_D$ $-44·1°$.

An acetyl derivative of the substance was prepared, and this, when crystallised from a mixture of ethyl acetate and alcohol, separated in flat needles, melting at 165—166°. It was analysed, and its optical rotatory power determined, with the following results:

0·1208 gave 0·3038 CO_2 and 0·0996 H_2O. C=68·6; H=9·2.

$C_{21}H_{34}O_2(CO\cdot CH_3)_2$ requires C=68·8; H=9·2 per cent.

0·3122, made up to 20 c.c. with chloroform, gave α_D $-0°49'$ in a 2-dcm. tube, whence $[\alpha]_D$ $-26·1°$.

These results render it evident that the above-described substance is identical with trifolianol (compare Power and Salway, this vol., p. 249).

The ethereal solution of the ethereal extract from which the trifolianol had been separated was evaporated, when 12 grams of a dark green resin were obtained. This was of an acidic nature, but yielded nothing of interest.

Chloroform, Ethyl Acetate, and Alcohol Extracts of the Resin.

These extracts were dark-coloured resins, amounting to 5, 2·5, and 8·3 grams respectively. They were thoroughly examined, but nothing definite could be isolated from them.

Summary.

The results of this investigation may be briefly summarised as follows:

The material employed consisted of the dried flowering tops of the carnation clover (*Trifolium incarnatum*, Linné). An alcoholic extract of this material, when distilled with steam, yielded an essential oil in an amount equivalent to 0·029 per cent. of the dried flowers. This essential oil, which contained furfuraldehyde, possessed a strong persistent odour, and the following constants: *d* 20°/20° = 0·9597; α_D $-1°48'$ in a 1-dcm. tube.

The portion of the alcoholic extract which was soluble in water contained a considerable quantity of a sugar which yielded *d*-phenylglucosazone (m. p. 205—206°), and from the aqueous liquid the following definite substances were isolated: benzoic and salicylic acids, in very small amount, with apparently a trace of *p*-coumaric acid; pratol, $C_{16}H_{12}O_4$; quercetin, $C_{15}H_{10}O_7$, and a new glucoside of the latter, $C_{21}H_{20}O_{12},3H_2O$ (m. p. 242—245°), which has been designated *incarnatrin*.

The portion of the alcoholic extract which was insoluble in water consisted chiefly of green, resinous material, the amount of which was equivalent to 4·1 per cent. of the weight of the dried flowers. From this resin the following compounds were obtained: an alcohol, $C_{34}H_{69}\cdot OH$ (m. p. 72—74°), which had previously been obtained from the wax of the humble-bee, and which it is now proposed to designate *incarnatyl alcohol*; hentriacontane, $C_{31}H_{64}$; a phytosterol, $C_{27}H_{46}O$ (m. p. 135—136°; $[\alpha]_D$ $-41·7°$), which yields an acetyl derivative melting at 124—125°, and having $[\alpha]_D$ $-43·9°$; trifolianol, $C_{21}H_{34}O_2(OH)_2$ (m. p. 295—300°; $[\alpha]_D$ $-44·1°$), and a mixture of fatty acids, consisting chiefly of palmitic, stearic, and linolic acids, with smaller amounts of oleic and *iso*linolenic acids.

In conclusion, the author wishes to express his indebtedness to Dr. F. B. Power for having suggested this research, and for the kind interest he has shown throughout the course of the work.

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