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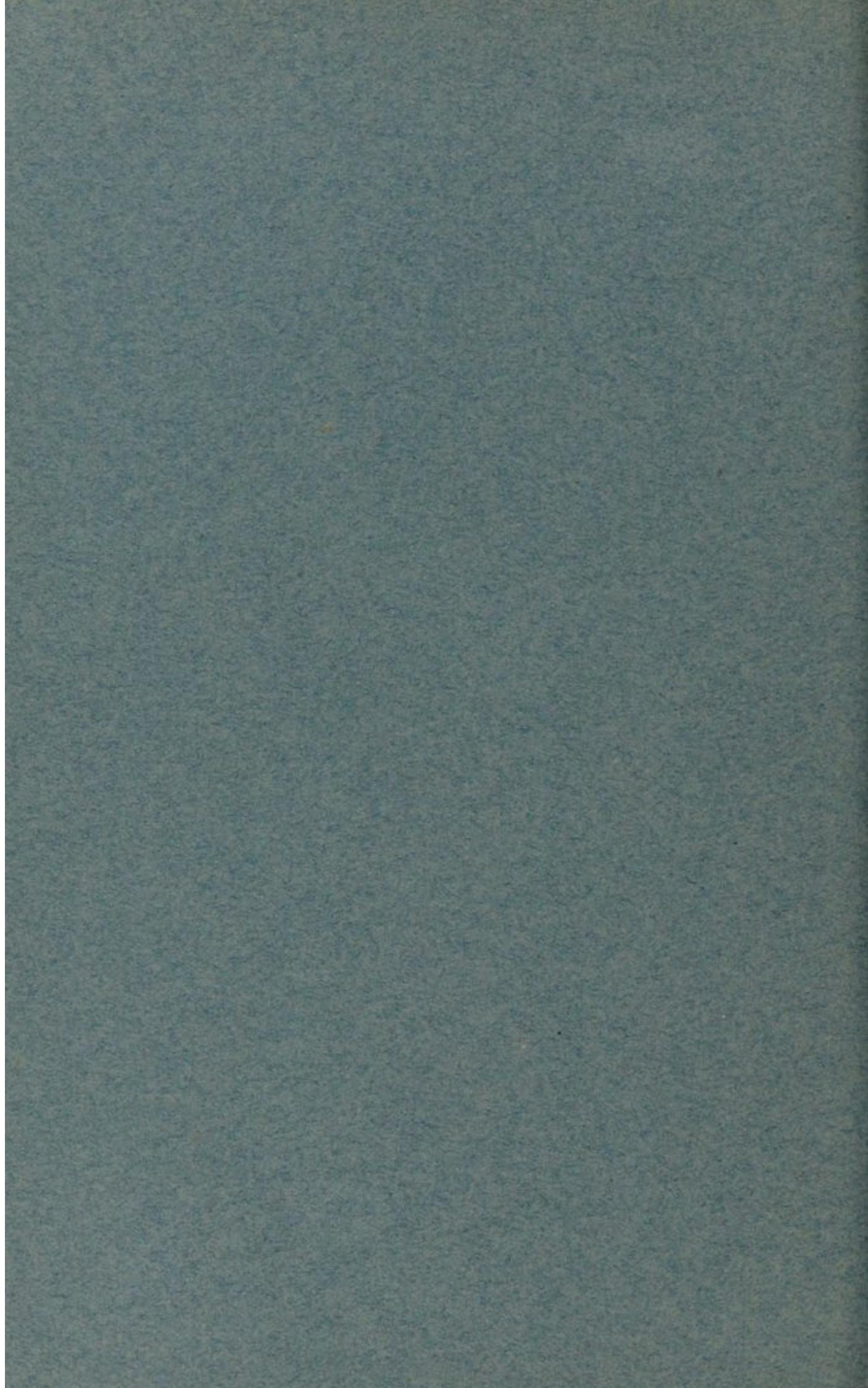
THE CONSTITUENTS
OF
RED CLOVER FLOWERS

BY
FREDERICK B. POWER, Ph.D.
AND
ARTHUR H. SALWAY, Ph.D.

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XXVI.—*The Constituents of Red Clover Flowers.*

By FREDERICK BELDING POWER and ARTHUR HENRY SALWAY.

THE flowers of the common red clover (*Trifolium pratense*, Linné) have been used to some extent medicinally in recent years on account of their assumed alterative properties, and have even been recommended in the treatment of cancer (*Amer. J. Pharm.*, 1881, 53, 85). So far as known to us, these flowers have never been subjected to a chemical examination, and the only knowledge of their constituents appears to be embodied in a statement that they contain tannin, two resins, fat, and chlorophyll. In this connexion it may incidentally be noted that Perkin and Phipps, in a paper entitled "Notes on some Natural Colouring Matters" (*Trans.*, 1904, 85, 58), have recorded that a cursory examination of the flowers of the white clover (*Trifolium repens*) indicated the presence of quercetin in the form of a glucoside. The same authors furthermore remark that "this colouring matter was recognised by the melting point of its acetyl derivative, and its decomposition products with caustic alkali, and in consequence of these observations a fuller investigation appeared unnecessary."

In view of the above considerations, and the fact that red clover flowers are available in practically unlimited quantities, it appeared desirable that a complete study of their constituents should be undertaken. The present investigation has disclosed the presence of a considerable number of new and interesting substances, and a summary of the results obtained is given at the end of this paper.

EXPERIMENTAL.

The material employed in this investigation was carefully collected for us under the superintendence of Mr. P. E. F. Perrédès, B.Sc., F.L.S., during the month of June, from a field of cultivated red clover (*Trifolium pratense*, Linné) in Kent. The flowers or blossoms were separately gathered, and were therefore, so far as possible, free from the green, herbaceous parts of the plant.

A portion (10 grams) of the dried flowers was tested for an alkaloid, but the reactions obtained were so slight as to indicate the presence of not more than traces of such a substance.

A further portion (25 grams) of the dried flowers was successively extracted in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried in a water-oven, were obtained:

Petroleum (b. p. 35—50°) extracted	0.50 gram	=	2.0 per cent.
Ether	0.50 "	=	2.0 "
Chloroform	0.35 "	=	1.4 "
Ethyl Acetate	1.50 "	=	6.0 "
Alcohol	4.50 "	=	18.0 "
Total	7.35 grams	=	29.4 per cent.

For the purpose of a complete examination, 264 kilograms of the flowers were collected. This material, after careful drying, amounted to 58.5 kilograms, or 22.16 per cent. of the original weight. The entire amount of this material was coarsely powdered, and then extracted by continuous percolation with hot alcohol. After the removal of the greater portion of the alcohol, which was conducted at a temperature not exceeding 85°, a dark green, viscid extract was obtained, which amounted to 15.78 kilograms.

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

A quantity (1.5 kilograms) of the above-mentioned extract was brought into a suitable apparatus with a little water, and steam passed through the mixture for several hours. The distillate, which contained some oily drops, was extracted with ether, the ethereal liquid being washed, dried, and the solvent removed. A small amount (1.5 grams) of an essential oil was thus obtained, which possessed a rather unpleasant odour. On subsequently treating 10 kilograms of the alcoholic extract in the manner above described, a further quantity (10.5 grams) of essential oil was obtained. The yield of oil was thus equivalent to 0.028 per cent. of the dried, or 0.006 per cent. of the fresh clover flowers. This essential oil, when distilled under diminished pressure, passed over between 90° and 160°/20 mm. as a light yellow liquid, which, however, on keeping, ultimately became reddish-brown, and was found to contain furfuraldehyde. It possessed the following constants: $d_{20}^{20} = 0.9476$; $\alpha_D + 4.0'$ in a 1-dcm. tube.

Non-volatile Constituents of the Extract.

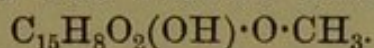
After the removal of the volatile constituents of the extract by distillation with steam, as above described, there remained in the

distillation flask a reddish-brown, aqueous liquid (A) and a quantity of a viscid resin (B), which became solid on cooling. The resin was collected, and thoroughly washed with water, the washings being added to the main portion of the aqueous liquid.

Examination of the Aqueous Liquid (A).

The aqueous liquid was concentrated under diminished pressure, and then repeatedly extracted with large volumes of ether. The ethereal extracts, which were of a light green colour, were united, washed with a little water, dried, and the ether removed, when 10 grams of a dark-coloured, soft solid were obtained. This was found by a preliminary examination to consist of a complex mixture of crystalline compounds, and, in order to obtain a quantity sufficient for their separation and characterisation, 10 kilograms of clover extract were treated as above described, the resulting aqueous liquid being extracted with ether. The yield of ethereal extract from this quantity of material was 64 grams.

Isolation of a New Phenolic Substance, Pratol,



The above-mentioned ethereal extract was digested with a quantity of ether insufficient to dissolve the whole, and the sparingly soluble portion, which amounted to 3 grams, separated by filtration. This product was repeatedly crystallised from alcohol, when a substance was obtained which separated in colourless needles, melting at 253° :

0.1265 gave 0.3307 CO_2 and 0.0513 H_2O . $\text{C}=71.3$; $\text{H}=4.5$.

0.1213 „ 0.3161 CO_2 „ 0.0500 H_2O . $\text{C}=71.1$; $\text{H}=4.6$.

$\text{C}_{16}\text{H}_{12}\text{O}_4$ requires $\text{C}=71.6$; $\text{H}=4.5$ per cent.

The above compound, when heated with acetic anhydride, yielded an *acetyl* derivative, which separated from alcohol in feathery needles, melting at 166° . This was analysed, and its molecular weight determined, with the following results:

0.1039 gave 0.2646 CO_2 and 0.0420 H_2O . $\text{C}=69.5$; $\text{H}=4.5$.

0.1069 „ 0.2715 CO_2 „ 0.0436 H_2O . $\text{C}=69.3$; $\text{H}=4.5$.

0.1485, in 33.26 of acetic acid, gave $\Delta t = 0.057^\circ$. $\text{M.W.}=305$.

$\text{C}_{16}\text{H}_{11}\text{O}_4(\text{CO}\cdot\text{CH}_3)$ requires $\text{C}=69.7$; $\text{H}=4.5$ per cent. $\text{M.W.}=310$.

The substance $\text{C}_{16}\text{H}_{12}\text{O}_4$ was found to contain one methoxyl group, as determined by Perkin's modification of the Zeisel method:

0.0995 gave 0.0785 AgI . $\text{MeO}=10.4$.

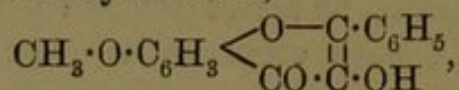
$\text{C}_{15}\text{H}_9\text{O}_3(\text{OMe})$ requires $\text{MeO}=11.6$ per cent.

No compound of the formula $\text{C}_{16}\text{H}_{12}\text{O}_4$, possessing the same

properties as that above described, appears to have hitherto been recorded. It is therefore proposed to designate the substance from red clover flowers as *pratol*, with reference to its phenolic character and the specific botanical name of the plant.

Pratol, $C_{15}H_8O_2(OH) \cdot O \cdot CH_3$, crystallises in a very characteristic form, since it separates from alcohol in needles, which, under the microscope, are seen to have a talon-like shape with curved edges. It is moderately soluble in hot alcohol, but only sparingly soluble in water, ether, chloroform, or benzene. It dissolves readily in hot aqueous sodium carbonate and sodium hydroxide, yielding pale yellow solutions. When dissolved in acetic anhydride, and a drop of sulphuric acid added, a yellow coloration is produced. With ferric chloride no appreciable change of colour was observed.

Pratol is isomeric with several flavone derivatives, such as the 2-methoxy- and 3-methoxy-flavonol,



prepared by Kostanecki and his co-workers (*Ber.*, 1904, **37**, 775; 1905, **38**, 993). The general behaviour of *pratol* is also very similar to that of the above-mentioned substances, and it therefore seems probable that it represents one of the many hydroxymethoxy-flavones which are theoretically possible.

Acetylpratol, $C_{16}H_{11}O_4(CO \cdot CH_3)$, prepared, as above noted, by heating *pratol* with acetic anhydride, is very soluble in hot alcohol, but only moderately so in chloroform or benzene.

The ethereal liquid from which the sparingly soluble *pratol* had been separated by filtration, as above described, was subsequently shaken with an aqueous solution of ammonium carbonate (*a*) until nothing further was removed by this reagent, then with successive small portions of aqueous sodium carbonate (*b*), and finally with aqueous sodium hydroxide (*c*). These extracts were then separately examined.

Isolation of Salicylic Acid.

The liquids obtained by extraction with ammonium carbonate (*a*) were united, acidified with sulphuric acid, and extracted with ether. This ethereal liquid, on the removal of the solvent, yielded a quantity (20 grams) of a dark green oil. The latter was digested with considerable quantities of hot light petroleum, these liquids being decanted, and the solvent evaporated. In this manner 2 grams of colourless needles were obtained, which, after a few crystallisations from water, melted at 154° and gave an intense violet coloration with ferric chloride:

0.1230 gave 0.2765 CO_2 and 0.0507 H_2O . $\text{C}=61.3$; $\text{H}=4.6$.

$\text{C}_7\text{H}_6\text{O}_3$ requires $\text{C}=60.9$; $\text{H}=4.3$ per cent.

This substance was thus identified as salicylic acid.

The portion of the ammonium carbonate extract which was insoluble in light petroleum was heated for some time with methyl alcohol in the presence of concentrated sulphuric acid. A product was thus obtained which could be further separated into phenolic and non-phenolic methyl esters by shaking its ethereal solution with dilute aqueous sodium hydroxide. The portion insoluble in the alkaline liquid, consisting of the non-phenolic esters, amounted to 10 grams, and was a dark-coloured oil. It was hydrolysed with alcoholic potassium hydroxide, and the regenerated acids distilled under diminished pressure, when fractions were collected at $100-130^\circ$ and $130-230^\circ/20$ mm., whilst a quantity of undistillable resin remained in the flask. These fractions were further examined by neutralising them with aqueous sodium carbonate, and fractionally precipitating the solutions with silver nitrate. A series of silver salts was thus obtained, which, with the exception of the last fraction (III), were more or less brown in colour. After drying in a vacuum over sulphuric acid, they were analysed:

Fraction $100-130^\circ/20$ mm.

0.1760 of salt gave 0.0852 Ag. $\text{Ag}=48.4$.

Fraction $130-230^\circ/20$ mm.

(I) 0.4969 of salt gave 0.1904 Ag. $\text{Ag}=38.4$.

(II) 0.1531 „ „ 0.0700 Ag. $\text{Ag}=45.7$.

(III) 0.7246 „ „ 0.4444 Ag. $\text{Ag}=61.3$.

It was evident from these results that the non-phenolic acids consisted of a complex mixture.

Identification of p-Coumaric Acid.

The portion of the above-mentioned methylated product which was soluble in aqueous sodium hydroxide was benzoylated by the Schotten-Baumann method, and then subjected to fractional crystallisation from alcohol. After a quantity of gummy, amorphous matter had been separated, a crystalline substance was deposited, which was ultimately obtained in slender needles, melting at 129° :

0.0783 gave 0.2079 CO_2 and 0.0390 H_2O . $\text{C}=72.4$; $\text{H}=5.5$.

$\text{BzO}\cdot\text{C}_6\text{H}_4\cdot\text{CH}\cdot\text{CH}\cdot\text{CO}_2\text{Me}$ requires $\text{C}=72.3$; $\text{H}=5.0$ per cent.

This compound is thus seen to agree in composition with the benzoyl derivative of methyl p-coumarate, which hitherto appears not to have been described. A little of it was therefore prepared for the purpose of comparison, when it was found to crystallise in

slender needles, melting at 129° , and to be identical in all respects with the compound obtained from the acid present in red clover flowers. The identity of the latter acid was further confirmed by the hydrolysis of the benzoyl derivative of its methyl ester, when a substance was obtained which crystallised from water in colourless needles, melting at 215° with evolution of carbon dioxide, and otherwise exhibited all the characters of *p*-coumaric acid.

Isolation of a New Yellow Compound, $C_{16}H_{10}O_7$.

The sodium carbonate extracts (*b*), obtained as above described, were separately acidified, and the resulting precipitates collected and examined. The product obtained from the first sodium carbonate extract consisted chiefly of chlorophyll, and nothing crystalline could be isolated from it. The second sodium carbonate extract yielded a yellow powder, which was dissolved in hot alcohol, in which it was freely soluble. This solution, after being kept for some time, deposited a small amount of a colourless, crystalline substance, which, after purification, melted at 253° , and yielded an acetyl derivative melting at 166° . It was found to be identical with the substance $C_{16}H_{12}O_4$, designated as pratol, which has already been described. As the mother liquors, which still contained all of the yellow compound, deposited nothing further on standing, the alcohol was completely removed, and the residue acetylated by means of acetic anhydride. On distilling off the greater part of the latter liquid, a crystalline product separated, which proved to be a mixture of substances. It was fractionally crystallised from alcohol, when a small quantity (1.0 gram) of an *acetyl* derivative was obtained, which appeared to be homogeneous, and separated in colourless, glistening, prismatic needles, melting at 145 — 147° with evolution of gas:

0.1530 gave 0.3347 CO_2 and 0.0555 H_2O . $C=59.7$; $H=4.0$.

The acetyl content of this compound was determined by heating it for some time with dilute sulphuric acid in the presence of alcohol, the alcohol being then completely removed, and the precipitated yellow substance, which was insoluble in cold water, collected and weighed:

0.1690 gave 0.1108 of yellow substance, or 65.6 per cent.

$C_{16}H_6O_7Ac_4$ requires $C=59.8$; $H=3.7$; $C_{16}H_{10}O_7=65.1$ per cent.

$C_{16}H_8O_7Ac_4$ „ $C=59.5$; $H=4.1$; $C_{16}H_{12}O_7=65.3$ „

It would appear from these results that the yellow compound possesses the formula $C_{16}H_{10}O_7$, and that it contains four hydroxyl groups. The presence of a methoxyl group was also established. The compound, $C_{16}H_{10}O_7$, as obtained from its acetyl derivative,

crystallises from boiling dilute alcohol in thin, yellow plates, which melt and decompose at about 280° . It is readily soluble in alcohol or acetic acid, but sparingly so in water, ether, chloroform, or benzene. It dissolves in alkalis with a yellow colour, and its solution in concentrated sulphuric acid exhibits a brilliant green fluorescence. In alcoholic solution it gives with ferric chloride a bluish-black coloration. The amount of this substance available was not sufficient for its further examination.

Isolation of a New Phenolic Substance, Pratensol, $C_{17}H_9O_2(OH)_3$.

The final sodium carbonate extract of the above-mentioned ethereal liquid yielded, on acidification, a light brown powder. This was dissolved in hot alcohol, and the solution kept for some time, when a small amount of the previously described pratol, $C_{16}H_{12}O_4$ (m. p. 253°), was deposited. The mother liquors from the latter contained a quantity of a very soluble substance, which could not be obtained directly in a crystalline form. The alcohol was therefore removed, and the residue acetylated, when a product was obtained which, after a few crystallisations from alcohol, yielded a pure, colourless *acetyl* derivative, melting at 189° :

0.1071 gave 0.2569 CO_2 and 0.0445 H_2O . $C=65.4$; $H=4.6$.

0.1002 „ 0.2410 CO_2 „ 0.0406 H_2O . $C=65.6$; $H=4.5$.

The molecular weight of the acetyl derivative was determined by the ebullioscopic method:

0.3518, in 25.62 of benzene, gave Δt 0.094° . M.W.=390.

$C_{23}H_{18}O_8$ requires $C=65.4$; $H=4.3$ per cent. M.W.=422.

The number of acetyl groups in this compound was ascertained by hydrolysing with aqueous sodium hydroxide, then adding a slight excess of sulphuric acid, and distilling the mixture in a current of steam:

0.2057 gave acetic acid equivalent to 14.45 c.c. $N/10$ -Ba(OH) $_2$.

$CO \cdot CH_3 = 30.2$.

$C_{17}H_9O_5(CO \cdot CH_3)_3$ requires $CO \cdot CH_3 = 30.6$ per cent.

From these results it may be concluded that the substance contained in red clover flowers, from which the above-described triacetyl derivative was prepared, possesses the formula $C_{17}H_{12}O_5$. In order to obtain the parent compound, the acetyl derivative was heated for some time in alcoholic solution with dilute sulphuric acid, and the alcohol subsequently removed by distillation with steam, when a colourless, crystalline substance separated. This was purified by crystallisation from benzene, when it separated in feathery needles, melting at 210° . Although the amount of this substance was insufficient for analysis, it was proved to represent

the original compound by the fact that when again acetylated, it yielded an acetyl derivative melting at 189° .

No compound of the formula $C_{17}H_{12}O_5$, possessing the same properties as that above described, appears to have hitherto been recorded. It is therefore proposed to designate the substance contained in red clover flowers as *pratensol*, with reference to its phenolic character and the specific botanical name of the plant.

Pratensol, $C_{17}H_9O_2(OH)_3$, is very readily soluble in alcohol and acetic acid, but only sparingly soluble in water, chloroform, and benzene. It dissolves in alkali carbonates, yielding yellow solutions, whilst its alcoholic solution gives with ferric chloride a greenish-black coloration.

Triacetylpratensol, $C_{17}H_9O_5(CO \cdot CH_3)_3$, crystallises from alcohol, in which it is only moderately soluble, in colourless, slender needles. It is readily soluble in glacial acetic acid, and moderately soluble in benzene. When warmed with dilute aqueous sodium hydroxide, not only are the three acetyl groups removed, but the molecule also undergoes further change, with the formation of a compound which crystallises from dilute alcohol in long, colourless needles, melting at $183-184^{\circ}$. If, on the other hand, the acetyl compound is heated with aqueous sodium carbonate, the product is a yellow, crystalline substance, which melts and decomposes at 160° . It was not possible to ascertain the exact nature of these changes on account of the small amount of substance available, since 37.1 kilograms of dried clover flowers yielded not more than 1.5 grams, or about 0.004 per cent., of pure triacetylpratensol.

Isolation of a New Phenolic Substance, $C_{15}H_7O_3(OH)_3$.

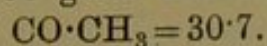
The sodium hydroxide extracts (c) of the previously mentioned ethereal liquid, when acidified, yielded a light green powder, which possessed an odour resembling that of eugenol. An attempt to identify the latter by distilling the product with steam was, however, unsuccessful. The light green powder was subsequently decolorised by treating its hot alcoholic solution with animal charcoal, when, on cooling, colourless clusters of feathery needles separated. After several crystallisations, the substance was obtained pure, and then melted at 225° :

0.0931 gave 0.2133 CO_2 and 0.0310 H_2O . $C=62.5$; $H=3.7$.

$C_{15}H_{10}O_6$ requires $C=62.9$; $H=3.5$ per cent.

This compound yielded an *acetyl* derivative, which separated from alcohol in thin, silky needles, melting at 209° . The acetyl content of this compound was ascertained by hydrolysing with dilute aqueous sodium hydroxide, then acidifying with sulphuric acid, and distilling the mixture in a current of steam:

0.1750 gave acetic acid equivalent to 12.5 c.c. $N/10\text{-Ba(OH)}_2$.



The molecular weight of the acetyl derivative was also ascertained by the ebullioscopic method:

0.1768, in 26.46 of benzene, gave Δt 0.043° . M.W. = 415.

$\text{C}_{15}\text{H}_7\text{O}_6(\text{CO}\cdot\text{CH}_3)_3$ requires $\text{CO}\cdot\text{CH}_3 = 31.3$ per cent. M.W. = 412.

The substance, $\text{C}_{15}\text{H}_{10}\text{O}_6$, is soluble in alkali hydroxides, forming a colourless solution. In alcoholic solution it gives with ferric chloride a dark green coloration. With concentrated sulphuric acid, only a faint yellow colour is produced.

The yield of the substance $\text{C}_{15}\text{H}_{10}\text{O}_6$ was very small, not more than 0.5 gram having been obtained from 37.1 kilograms of dried clover flowers.

Isolation of a New Glucoside, Trifolin, $\text{C}_{22}\text{H}_{22}\text{O}_{11}\cdot\text{H}_2\text{O}$.

The original aqueous liquid (A), which had been extracted with ether, as already described, was kept for a considerable time, when it was observed that a quantity (about 60 grams) of a light brown solid had been deposited. This was collected, and purified by repeated crystallisation from aqueous pyridine, when it separated in minute, slender, pale yellow needles, melting and decomposing at about 260° . The substance contained water of crystallisation, which was lost at 115° , but was again absorbed on exposing the anhydrous substance to the air:

0.3051, on heating at 115° , lost 0.0130 H_2O . $\text{H}_2\text{O} = 4.3$.

0.1101 * gave 0.2302 CO_2 and 0.0464 H_2O . $\text{C} = 57.0$; $\text{H} = 4.7$.

0.1194 * „ 0.2481 CO_2 „ 0.0506 H_2O . $\text{C} = 56.7$; $\text{H} = 4.7$.

0.1010 * „ 0.2115 CO_2 „ 0.0440 H_2O . $\text{C} = 57.1$; $\text{H} = 4.8$.

$\text{C}_{22}\text{H}_{22}\text{O}_{11}\cdot\text{H}_2\text{O}$ requires $\text{H}_2\text{O} = 3.7$ per cent.

$\text{C}_{22}\text{H}_{22}\text{O}_{11}$ requires $\text{C} = 57.1$; $\text{H} = 4.8$ per cent.

These results indicated the formula of the above-described substance to be $\text{C}_{22}\text{H}_{22}\text{O}_{11}$, and it proved to be a glucoside. As it is a new compound, it is proposed to designate it *trifolin*, with reference to the generic name of the plant from which it was obtained.

Trifolin, $\text{C}_{22}\text{H}_{22}\text{O}_{11}\cdot\text{H}_2\text{O}$, is insoluble in chloroform, benzene, or cold water. It is not readily dissolved by alcohol, but is very soluble in pyridine and is best crystallised from a mixture of the latter solvent with water. With aqueous sodium carbonate and the alkali hydroxides, it gives intensely yellow solutions. It dissolves in concentrated sulphuric acid, forming at first a yellow solution, but which rapidly develops a brilliant green fluorescence. In alcoholic solution it gives with ferric chloride a dark brown coloration. No crystalline acetyl derivative of trifolin could be prepared.

* Anhydrous substance.

Hydrolysis of Trifolin: Formation of a Yellow Colouring Matter, Trifolitin, $C_{16}H_{10}O_6$.

Trifolin was dissolved in alcohol, a little dilute sulphuric acid added, and the mixture heated on the water-bath for an hour. The alcohol was then removed by distillation in a current of steam, when a yellow, crystalline substance separated. This was collected, and washed with cold water, the filtrate having been set aside for the subsequent examination of the sugar. Some difficulty was experienced in satisfactorily crystallising the yellow hydrolytic product, for it is extremely soluble in alcohol, and invariably separates from dilute alcohol at the ordinary temperature in an apparently amorphous condition. On the other hand, if the alcohol is removed from the solution at the boiling temperature, the substance then separates from the hot liquid in thin, yellow needles. It is, however, best purified by crystallisation from moist nitrobenzene, when it separates in clusters of slender, yellow needles, which melt and decompose at about 275° . After drying at 130° , it was analysed:

0.0786 gave 0.1846 CO_2 and 0.0264 H_2O . $C=64.1$; $H=3.7$.

$C_{16}H_{10}O_6$ requires $C=64.4$; $H=3.4$ per cent.

By heating the yellow hydrolytic product with acetic anhydride an *acetyl* derivative was obtained. The latter, when crystallised from alcohol, separated in colourless, silky needles, which, when rapidly heated, melted at about 116° , then resolidified, and finally melted at 182° . This behaviour was due to the presence of water of crystallisation, the melting point of the anhydrous substance being 182° :

0.5676, on heating at 105° , lost 0.0231 H_2O . $H_2O=4.1$.

$C_{16}H_6O_6(CO \cdot CH_3)_4 \cdot H_2O$ requires $H_2O=3.7$ per cent.

The anhydrous substance was analysed, and its molecular weight determined, with the following results:

0.1078 gave 0.2426 CO_2 and 0.0401 H_2O . $C=61.4$; $H=4.1$.

0.1056 „ 0.2375 CO_2 „ 0.0391 H_2O . $C=61.3$; $H=4.1$.

0.4012, in 28.14 of phenol, gave $\Delta t = 0.216^{\circ}$. M.W.=495.

$C_{16}H_6O_6(CO \cdot CH_3)_4$ requires $C=61.8$; $H=3.9$ per cent. M.W.=466.

In order to ascertain the number of acetyl groups in the above compound, it was dissolved in alcohol, dilute sulphuric acid added, and the mixture heated for several hours on the water-bath. The alcohol was then completely removed by distillation in a current of steam, and, after cooling, the precipitated yellow substance was collected and weighed, it having been found that the amount dissolved by the water was negligible:

0.1811 gave 0.1160 of colouring matter, or 64.1 per cent.

0.2922 „ 0.1876 „ „ 64.2 „

$C_{16}H_6O_6(CO \cdot CH_3)_4$ requires $C_{16}H_{10}O_6 = 63.9$ per cent.

The yellow colouring matter, as obtained from the above-described acetyl derivative, was crystallised from dilute alcohol, dried at 120° , and again analysed:

0.0855 gave 0.2000 CO_2 and 0.0276 H_2O . $C = 63.8$; $H = 3.6$.

$C_{16}H_{10}O_6$ requires $C = 64.4$; $H = 3.4$ per cent.

It is evident from the above results that the yellow colouring matter obtained by the hydrolysis of the glucoside trifolin possesses the formula $C_{16}H_{10}O_6$, and that it contains four hydroxyl groups. As no compound of this formula, having the same properties, has hitherto been recorded, it is proposed to designate it *trifolitin*.

Trifolitin, $C_{16}H_{10}O_6$, is readily soluble in alcohol and glacial acetic acid, but only very sparingly soluble in benzene, chloroform, or ether. It dissolves in alkalis with an intense yellow colour, and dyes mordanted cotton wool a bright yellow. When dissolved in concentrated sulphuric acid, it gives a yellow solution, followed by a brilliant green fluorescence. It is precipitated from its alcoholic solution by basic lead acetate as an orange-yellow lead salt. In alcoholic solution it gives with ferric chloride a dark green coloration. Trifolitin is not altered when heated for several hours with 30 per cent. aqueous potassium hydroxide, and it contains no methoxyl group. It is evident from its empirical formula, $C_{16}H_{10}O_6$, that it cannot belong to the flavone group of natural colouring matters, and it differs, moreover, from the flavone compounds by the fact that it forms no salt with sulphuric acid (Trans., 1896, 80, 206), whilst it is only with difficulty that a potassium salt is deposited from its concentrated alcoholic solution on the addition of potassium acetate. On the other hand, the composition and general properties of trifolitin would agree with those of a tetra-hydroxy-derivative of phenylnaphthaquinone, but the amount of material available was not sufficient to enable us definitely to ascertain the group of naturally occurring dyes to which it belongs.

The aqueous liquid obtained in the course of hydrolysis of the glucoside trifolin was treated with barium carbonate for the removal of the sulphuric acid, and evaporated to the consistency of a syrup. This liquid readily reduced Fehling's solution, and yielded a crystalline osazone which, in the crude condition, melted and decomposed at 180° . When crystallised from aqueous pyridine, it separated in needles, which melted and decomposed at 194° :

0.1258 gave 0.2902 CO_2 and 0.0738 H_2O . $C = 62.9$; $H = 6.5$.

$C_{18}H_{22}O_3N_4$ requires $C = 63.2$; $H = 6.4$ per cent.

The above result indicated that the sugar formed by the

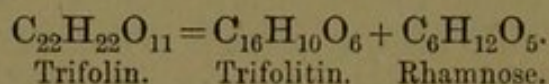
hydrolysis of trifolin has the formula $C_6H_{12}O_5$, and it appeared to be rhamnose, the osazone of which, however, is stated to melt at 180° . For the purpose of comparison, an osazone was prepared from a commercial specimen of rhamnose. This, when crystallised from aqueous pyridine, was found to melt and decompose at 188° , and appeared to be identical with that above described. Trifolin is therefore converted by hydrolysis into trifolitin and rhamnose. A known weight of the glucoside was subsequently hydrolysed by dilute sulphuric acid in aqueous alcohol, the alcohol then removed by passing steam through the mixture, and, after cooling, the precipitated trifolitin was collected, washed with cold water, dried at 120° , and weighed:

0.2056 gave 0.1318 of hydrolytic product, or 64.1 per cent.

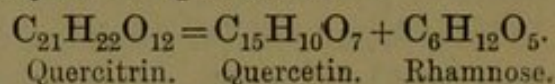
0.2885 „ 0.1859 „ „ 64.4 „

$C_{22}H_{22}O_{11}$ requires $C_{16}H_{10}O_6 = 64.5$ per cent.

The hydrolysis of trifolin therefore takes place according to the following equation:



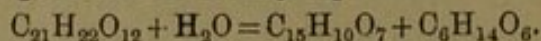
This equation would appear to indicate that the formation of trifolitin and rhamnose from trifolin is unaccompanied by the absorption of water, and would thus be analogous to that representing the hydrolysis of quercitrin*:



The explanation of this apparent anomaly may be, either that these glucosides tenaciously retain a molecule of water, or that the respective phenolic hydrolytic products suffer dehydration immediately after their formation.

The dark-coloured aqueous liquid from which the above-described glucoside, trifolin, had separated was next shaken repeatedly with hot amyl alcohol. The amyl alcohol extracts were united, washed with a little water, and then concentrated under diminished pressure, when, on cooling, a yellow, gelatinous substance was deposited. This was removed by filtration, and the filtrate again concentrated, when a further quantity of a yellow substance was obtained. To the final amyl-alcoholic filtrate, light petroleum was added, which

* The hydrolysis of quercitrin is usually represented as follows:



This equation, however, is evidently incorrect, inasmuch as it is now known that rhamnose possesses the formula $C_6H_{12}O_5$, but crystallises with one molecule of water (compare A. G. Perkin, *J. Soc. Chem. Ind.*, 1903, 22, 602, and Brauns, *Arch. Pharm.*, 1904, 242, 561).

precipitated the remainder of the yellow substance as an amorphous powder. The three fractions thus obtained were separately examined.

Fraction I.—This product consisted of a yellow, highly hygroscopic substance. It was dissolved in hot alcohol, and the solution kept for some time, when a gelatinous, brown mass separated. The latter was twice subjected to the preceding treatment with alcohol, when a crystalline substance was obtained, which was finally purified by crystallisation from dilute alcohol. It then formed colourless, glistening leaflets, melting at 214° . The amount of pure substance isolated was 0.5 gram:

0.0989 gave 0.2199 CO_2 and 0.0440 H_2O . $\text{C}=60.6$; $\text{H}=4.9$.

0.1154 „ 0.2559 CO_2 „ 0.0496 H_2O . $\text{C}=60.5$; $\text{H}=4.8$.

$\text{C}_{14}\text{H}_{12}\text{O}_6$ requires $\text{C}=60.9$; $\text{H}=4.3$ per cent.

This substance would thus appear to possess the formula $\text{C}_{14}\text{H}_{12}\text{O}_6$, and, as it differs in its properties from any substance of this formula which has hitherto been recorded, it may be regarded as a new compound.

The compound, $\text{C}_{14}\text{H}_{12}\text{O}_6$, is insoluble in water, chloroform, or benzene, but readily soluble in hot alcohol. It is insoluble in aqueous sodium carbonate, but readily dissolves in alkali hydroxides without change of colour. It gives no coloration with concentrated sulphuric acid, and is not changed by heating with dilute sulphuric acid. Its alcoholic solution yields with ferric chloride a light green coloration.

Isolation of a Second Glucoside of Trifolitin, isoTrifolin, $\text{C}_{22}\text{H}_{22}\text{O}_{11}$.

Fraction II.—This product, consisting of a light yellow powder, was fractionally crystallised from alcohol. The more soluble portion contained a substance which separated from very dilute alcohol in pale yellow needles, melting and decomposing at about 250° with the formation of a deep red liquid. It was dried at 115° :

0.1100 gave 0.2282 CO_2 and 0.0451 H_2O . $\text{C}=56.6$; $\text{H}=4.6$.

$\text{C}_{22}\text{H}_{22}\text{O}_{11}$ requires $\text{C}=57.1$; $\text{H}=4.8$ per cent.

The glucosidic character of this substance was proved by heating with dilute sulphuric acid in aqueous alcohol. On removing the alcohol, a yellow colouring matter separated from the hot solution in thin needles, which melted and decomposed at 275° . This hydrolytic product yielded an acetyl derivative, which was found to be identical with that prepared from the previously described trifolitin, $\text{C}_{16}\text{H}_{10}\text{O}_6$. The aqueous liquid obtained in the course of the above hydrolysis readily reduced Fehling's solution, but the amount of glucoside available (0.5 gram) did not permit of preparing an osazone from the sugar.

Although the above-described glucoside yields the same yellow hydrolytic product, and possesses apparently the same empirical formula as the glucoside trifolin, the two compounds are evidently not identical. Thus trifolin (m. p. 260°) separates from dilute alcohol in small, globular aggregates of fine needles, whilst the glucoside, melting at 250° , is much more soluble, and crystallises in clusters of comparatively large needles. It is therefore proposed to designate the second glucoside of trifolitin as *isotrifolin*.

The general behaviour of *isotrifolin*, as might be expected, is very similar to that of trifolin. It dissolves in alkalis with the formation of a deep yellow solution. With concentrated sulphuric acid, a yellow coloration is produced, together with a green fluorescence, and in alcoholic solution it gives with ferric chloride a deep brown colour.

Isolation of a Glucoside of Quercetin.

The sparingly soluble portion of the above-described fraction II was finally purified by crystallisation from water, when it separated in pale yellow, prismatic needles, melting and decomposing at 235° :

0.1484, on heating at 110° , lost 0.0161 H_2O . $\text{H}_2\text{O}=10.8$.

0.1117, dried at 110° , gave 0.2184 CO_2 and 0.0475 H_2O .

$\text{C}=53.3$; $\text{H}=4.7$.

The substance dissolved readily in hot water, giving a yellow solution, the colour of which was greatly intensified by the addition of alkalis. With concentrated sulphuric acid, a green fluorescence was produced. Its aqueous solution gave with ferric chloride a dark green coloration.

On heating the substance for a short time in aqueous solution with dilute sulphuric acid, a crystalline, yellow compound separated, and the filtrate from the latter readily reduced Fehling's solution. It was thus evident that the substance was a glucoside. The yellow hydrolytic product melted and decomposed at 305° , and, by means of its acetyl derivative (m. p. 195°), was identified as quercetin. The amount of this glucoside obtained from 37.1 kilograms of dried red clover flowers was only about 0.5 gram, and it was therefore not possible more definitely to characterise it.

Fraction III.—This was a yellow, amorphous solid, which was freely soluble in alcohol, giving a dark-coloured solution. The attempts to obtain something crystalline from this solution were unsuccessful, and as a preliminary test indicated the presence of glucosidic substances, the whole of the fraction, together with the mother liquors from fractions I and II, was hydrolysed by heating for some time with dilute sulphuric acid in aqueous alcohol. After removing the alcohol, a quantity of a dark-coloured solid separated,

which was collected and washed with water. This product was found to be a highly complex mixture, which, however, by digestion with ether, could be separated into readily and sparingly soluble portions. The ethereal solution of the readily soluble portion was extracted with alkalis, when it yielded traces of salicylic acid, a small amount of pratol, $C_{16}H_{12}O_4$, and a yellow compound melting at 305° , which, by means of its acetyl derivative (m. p. 194°), was identified as quercetin.

Isolation of isoRhamnetin, $C_{16}H_{12}O_7$.

The portion of the above-mentioned hydrolytic product which was sparingly soluble in ether was treated with aqueous sodium carbonate, when it was found to be only partly soluble in the alkaline liquid. From the insoluble portion a small amount of the previously described compound, $C_{14}H_{12}O_6$ (m. p. 214°), was obtained, whilst the portion soluble in the alkali carbonate consisted chiefly of a yellow colouring matter, which was observed to differ from any of the substances already isolated from the clover flowers. This substance was sparingly soluble in acetic acid and alcohol, and was purified by conversion into its acetyl derivative, the latter being then fractionally crystallised from alcohol until constant in melting point. It then separated in thin, hair-like needles, melting at $198-200^\circ$. After being dried at 120° :

0.0927 gave 0.2017 CO_2 and 0.0354 H_2O . $C=59.3$; $H=4.2$.

$C_{16}H_8O_7(CO \cdot CH_3)_4$ requires $C=59.5$; $H=4.1$ per cent.

The number of acetyl groups in this derivative was determined by hydrolysis with dilute sulphuric acid, the hydrolytic product being then collected and weighed:

0.1120 gave 0.0722 of colouring matter, or 64.5 per cent.

0.1126 „ 0.0734 „ „ 65.2 „

$C_{16}H_8O_7(CO \cdot CH_3)_4$ requires $C_{16}H_{12}O_7=65.3$ per cent.

The hydrolytic product, which melted and decomposed at about 295° , was a deep yellow substance, only sparingly soluble in alcohol or acetic acid, and insoluble in chloroform or benzene. It dissolved in alkalis, with the production of a deep yellow colour, and its solution in sulphuric acid possessed a green fluorescence. It was found to contain one methoxyl group, as determined by Perkin's modification of the Zeisel method:

0.0941 of acetyl compound gave 0.0379 AgI. $MeO=5.3$.

$C_{15}H_5O_6(OCH_3)(CO \cdot CH_3)_4$ requires $MeO=6.4$ per cent.

From these analytical data it may be concluded that the yellow substance has the empirical formula $C_{16}H_{12}O_7$, and that it contains four hydroxyl groups and one methoxyl group. The composition

and characters of this substance are thus in agreement with those of *isorhamnetin*, a colouring matter which was first isolated by A. G. Perkin (Trans., 1896, **69**, 1658) from the petals of the yellow wallflower (*Cheiranthus Cheiri*). The last-mentioned author had, however, recorded the melting point of tetra-acetyl*isorhamnetin* as 195—196°.

In order to further confirm the identity of the substance contained in red clover flowers with *isorhamnetin*, the product resulting from the methoxyl determination was examined. This separated from the acid solution, on cooling, in yellow, silky needles, which did not melt, but partially volatilised, at a temperature below 300°. It gave an acetyl derivative melting at 190—192°, and possessed the general characters of quercetin, thus affording conclusive evidence that the respective substance in red clover flowers was a monomethyl derivative of quercetin and identical with *isorhamnetin*.

The aqueous liquid which had been extracted with hot amyl alcohol, as above described, was dark brown in colour, and gave an abundant yellow precipitate on the addition of basic lead acetate. This precipitate was examined in the usual way, but, with the exception of a small amount of yellow colouring matter, nothing definite could be isolated from it. The filtrate from the basic lead acetate precipitate, after removal of the excess of lead, was of a bright yellow colour, and readily reduced Fehling's solution. It contained a large quantity of sugar, which yielded *d*-phenylglucosazone, melting at 205°. The liquid was concentrated under diminished pressure to the consistency of a syrup, and kept for a considerable time, but nothing crystalline separated from it.

The Resins (B).

The resinous material obtained from 1.5 kilograms of the original alcoholic extract of red clover flowers was a dark green, soft solid, and amounted to 314 grams. It was dissolved in alcohol, the solution mixed with purified sawdust, and the thoroughly dried mixture then successively extracted in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 100°, were obtained:

Petroleum (b. p. 30—45°) extracted	190.0 grams	=	60.5 per cent.
Ether	19.0	=	6.1
Chloroform	6.7	=	2.1
Ethyl Acetate	10.3	=	3.3
Alcohol	43.0	=	13.7

Total 269.0 grams = 85.7 per cent.

Petroleum Extract of the Resin.

This extract consisted of a dark green, semi-solid fat. It was hydrolysed by heating for a short time with an alcoholic solution of potassium hydroxide, after which the alcohol was removed, water added, and the alkaline mixture repeatedly extracted with ether. The ethereal extracts were united, washed, dried, and the solvent removed, when a quantity (46.5 grams) of a brownish-yellow solid was obtained.

Isolation of Myricyl Alcohol, $C_{31}H_{63}\cdot OH$.

The above-mentioned, brownish-yellow, unsaponifiable material was agitated with cold alcohol, and thus separated into a soluble and an insoluble portion. The latter consisted of a colourless, wax-like solid, melting somewhat indefinitely at 70—74°. It appeared to consist of a mixture of several substances, and was therefore subjected to prolonged fractional crystallisation from a mixture of ethyl acetate and alcohol. In this manner the least soluble constituent was ultimately obtained pure, and then melted at 85°. It crystallised from ethyl acetate in small, colourless needles, whilst from alcohol it separated in glistening leaflets:

0.1250 gave 0.3776 CO_2 and 0.1597 H_2O . $C=82.4$; $H=14.2$.

$C_{31}H_{64}O$ requires $C=82.3$; $H=14.2$ per cent.

The analysis and properties of this substance establish its identity as myricyl alcohol.

Isolation of Heptacosane, $C_{27}H_{56}$, Hentriacontane, $C_{31}H_{64}$, and Sitosterol, $C_{27}H_{48}O$.

The more soluble substances obtained in the course of the above fractionation could not be further separated by crystallisation. A preliminary examination having indicated that they consisted chiefly of hydrocarbons, with presumably a little myricyl alcohol, the mixture was heated at 130° for an hour with an equal weight of phthalic anhydride, in order to convert the myricyl alcohol into its acid phthalic ester, and thus effect its removal. The product was subsequently digested with a mixture of ether and chloroform, the solution filtered, and shaken with aqueous sodium carbonate. An insoluble compound thus separated, which was collected, and found to consist of myricyl sodium phthalate. The ether-chloroform solution was then evaporated, and the residue heated with aqueous sodium hydroxide in order to remove unchanged phthalic anhydride. The portion insoluble in the alkali was afterwards dissolved in chloroform, the solution being washed, dried, and the solvent

removed. A wax-like solid was thus obtained, which was subjected to fractional crystallisation from ethyl acetate. The most sparingly soluble fraction, which separated in pearly leaflets, melted at 65° , and this melting point was not changed by further crystallisation:

0.1001 gave 0.3121 CO_2 and 0.1324 H_2O . $\text{C}=85.0$; $\text{H}=14.7$.

$\text{C}_{31}\text{H}_{64}$ requires $\text{C}=85.3$; $\text{H}=14.7$ per cent.

This substance was evidently hentriacontane (m. p. 68°), the somewhat low melting point being probably due to the presence of a small amount of the following hydrocarbon.

From the more readily soluble fractions, a substance was isolated, which separated in pearly leaflets, melting at $58-59^{\circ}$:

0.0944 gave 0.2943 CO_2 and 0.1229 H_2O . $\text{C}=85.0$; $\text{H}=14.5$.

$\text{C}_{27}\text{H}_{56}$ requires $\text{C}=85.3$; $\text{H}=14.7$ per cent.

This substance was thus identified as heptacosane.

The portion of the unsaponifiable material which was soluble in cold alcohol amounted to 30.2 grams. It was twice distilled under diminished pressure, and the following five fractions collected: $200-210^{\circ}$; $210-220^{\circ}$; $220-290^{\circ}$; $290-300^{\circ}$; and above $300^{\circ}/20$ mm.

Fraction $200-210^{\circ}/20$ mm.—This was a pale yellow liquid, and contained a considerable quantity of an unsaturated substance:

0.1083 gave 0.3256 CO_2 and 0.1289 H_2O . $\text{C}=82.0$; $\text{H}=13.2$.

0.1234 absorbed 0.1008 iodine. Iodine value = 81.7.

Fraction $210-220^{\circ}/20$ mm.—This was a brownish-yellow liquid, and, like the preceding fraction, was unsaturated:

0.1064 gave 0.3141 CO_2 and 0.1288 H_2O . $\text{C}=80.5$; $\text{H}=13.5$.

0.0990 absorbed 0.0832 iodine. Iodine value = 84.0.

Both the above fractions were evidently mixtures, and nothing of a definite nature could be obtained from them.

Fractions $220-290^{\circ}$; $290-300^{\circ}$; and above $300^{\circ}/20$ mm.—These fractions solidified on cooling, and were found to consist chiefly of a phytosterol, with a small quantity of the previously mentioned hydrocarbons. After repeated crystallisation, a substance was isolated which separated from a mixture of ethyl acetate and alcohol in colourless, glistening leaflets, melting at $135-136^{\circ}$:

0.3194, on heating at 110° , lost 0.0160 H_2O . $\text{H}_2\text{O}=5.0$.

$\text{C}_{27}\text{H}_{46}\text{O}, \text{H}_2\text{O}$ requires $\text{H}_2\text{O}=4.5$ per cent.

0.0963, dried at 110° , gave 0.2959 CO_2 and 0.1033 H_2O . $\text{C}=83.8$; $\text{H}=11.9$.

$\text{C}_{27}\text{H}_{46}\text{O}$ requires $\text{C}=83.9$; $\text{H}=11.9$ per cent.

0.2244 of anhydrous substance, made up to 25 c.c. with chloroform, gave $\alpha_D - 0.37'$ in a 2 dcm. tube, whence $[\alpha]_D - 34.4^{\circ}$.

The above-described substance was evidently a phytosterol, and it gave the colour reactions of this class of compounds. It yielded an acetyl derivative, melting at 126° , and therefore is identical with sitosterol (*Monatsh.*, 1897, **18**, 551).

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

The aqueous alkaline solution of potassium salts resulting from the hydrolysis of the petroleum extract of the resin, having been shaken with ether to remove unsaponifiable material, as already described, was acidified with dilute sulphuric acid, when a dark-coloured semi-solid precipitate of fatty acids was produced. On extracting the mixture with ether, however, it was observed that a portion of the precipitate was very sparingly soluble in that liquid. The sparingly soluble material was therefore collected, when it formed a pale green solid, amounting to 3 grams. It was purified by crystallisation from pyridine containing a little alcohol, from which it was deposited in minute, colourless needles, melting and decomposing at 295° . After being dried at 110° :

0.1049 gave 0.2746 CO_2 and 0.0982 H_2O . $C=71.4$; $H=10.4$.

$C_{21}H_{36}O_4$ requires $C=71.6$; $H=10.2$ per cent.

This compound yielded an *acetyl* derivative, which separated from alcohol in flat, colourless needles, melting at $165-166^{\circ}$. An analysis and the determination of its optical rotatory power gave the following results:

0.0968 gave 0.2442 CO_2 and 0.0799 H_2O . $C=68.8$; $H=9.2$.

$C_{21}H_{34}O_4(CO \cdot CH_3)_2$ requires $C=68.8$; $H=9.2$ per cent.

0.2435, made up to 25 c.c. with chloroform, gave $\alpha_D -0^{\circ}30'$ in a 2-dcm. tube, whence $[\alpha]_D -25.7^{\circ}$.

From the above results it was evident that the compound melting at 295° is a dihydric alcohol, having the empirical formula $C_{21}H_{36}O_4$. As it is a new substance, it is proposed to designate it *trifolianol*, with reference to the generic name of the plant from which it was obtained.

Trifolianol, $C_{21}H_{34}O_2(OH)_2$, is practically insoluble in water, and very sparingly soluble in alcohol, ether, and chloroform, but readily soluble in dilute pyridine, from which it crystallises in minute needles. When dissolved in chloroform with a little acetic anhydride, and a drop of concentrated sulphuric acid subsequently added, it gives a transient pink coloration, changing to blue, and finally to green. The properties of trifolianol are similar to those of two other dihydric alcohols which have recently been isolated in these laboratories, namely, ipuranol, $C_{23}H_{38}O_2(OH)_2$ (*Amer. J. Pharm.*, 1908, **80**, 264, 576; *Trans.*, 1908, **93**, 907; 1909, **95**, 249),

and citrullol, $C_{22}H_{36}O_2(OH)_2$ (this vol., p. 102). These alcohols therefore appear to be members of a homologous series, which is represented by the general formula, $C_nH_{2n-6}O_4$.

Examination of the Fatty Acids.

The ethereal solution, from which the above-described trifolanol had been removed by filtration, was washed, dried, and the solvent removed. The mixture of fatty acids thus obtained contained a considerable quantity of resinous material, which was insoluble in light petroleum, and was therefore removed by digesting the mixture with the latter solvent, and filtering. This resin was a dark green solid, amounting to 10 grams, but no definite crystalline compound could be isolated from it. The petroleum solution, on evaporation, yielded a dark-coloured mixture of fatty acids, which was distilled under diminished pressure. The pale yellow distillate, amounting to 60 grams, became partly solid on cooling. It was dissolved in alcohol, when a substance separated which, after several crystallisations from alcohol, melted at $60-61^\circ$:

0.1182 gave 0.3236 CO_2 and 0.1341 H_2O . $C=74.7$; $H=12.6$.

0.1280 required for neutralisation 5.02 c.c. $N/10-KOH$.

Neutralisation value = 220.

$C_{16}H_{32}O_2$ requires $C=75.0$; $H=12.5$ per cent.

Neutralisation value = 219.1.

This substance was thus identified as palmitic acid.

In order to ascertain the nature of the remaining constituents of the mixed fatty acids, the mother liquors from the above crystallisations were united, and, while hot, fractionally precipitated with a concentrated aqueous solution of barium acetate. The final precipitate was oily, and, as it consisted for the most part of the barium salts of unsaturated acids, it was separately examined as described below. From the other fractions of barium salt the fatty acids were regenerated, and, after one crystallisation from acetic acid, were titrated with a decinormal solution of potassium hydroxide. The neutralisation values of the several fractions were 199.0; 210.0; 213.0; 213.5; 214.8; and 216.9, whilst their melting points were $53-55^\circ$; $53-56^\circ$; $53-56^\circ$; $53-56^\circ$; $54-56^\circ$; and $56-59^\circ$ respectively. These results indicated that the saturated acids consisted chiefly of a mixture of palmitic and stearic acids, the respective neutralisation values of which are 219.1 and 197.7.

The acids obtained from the above-mentioned oily barium salt were converted into their lead salts, and the latter treated with ether. From the portion of lead salt which was undissolved by the ether, a further small amount of palmitic acid was obtained. The acids regenerated from the soluble lead salt formed a pale

yellow oil, which was distilled under diminished pressure. An analysis and the determination of its constants gave the following results:

0.1428 gave 0.4031 CO_2 and 0.1437 H_2O . $\text{C}=77.0$; $\text{H}=11.2$.
 $d\ 20^\circ/20^\circ=0.9148$. Neutralisation value 200.9. Iodine value 184.7.
 $\text{C}_{18}\text{H}_{34}\text{O}_2$ requires $\text{C}=76.6$; $\text{H}=12.1$ per cent. Neutralisation value=198.9. Iodine value=90.1.
 $\text{C}_{18}\text{H}_{32}\text{O}_2$ requires $\text{C}=77.1$; $\text{H}=11.4$ per cent. Neutralisation value=200.4. Iodine value =181.4.
 $\text{C}_{18}\text{H}_{30}\text{O}_2$ requires $\text{C}=77.7$; $\text{H}=10.8$ per cent. Neutralisation value=201.8. Iodine value=274.0.

In order to ascertain more definitely the nature of the unsaturated acids, 10 grams of the mixture were dissolved in alkali, and oxidised with a 1.5 per cent. solution of potassium permanganate. The chief product of the oxidation, when crystallised from water, separated in thin needles, melting at $156-157^\circ$:

0.0630 gave 0.1426 CO_2 and 0.0594 H_2O . $\text{C}=61.7$; $\text{H}=10.5$.
 $\text{C}_{18}\text{H}_{36}\text{O}_6$ requires $\text{C}=62.1$; $\text{H}=10.3$ per cent.

This substance was evidently tetrahydroxystearic acid (sativic acid). In addition to the latter acid, very small quantities of dihydroxystearic acid (m. p. 130°), linusic acid (m. p. $200-205^\circ$), and isolinusic acid (m. p. $173-174^\circ$) were obtained.

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

Ethereal Extract of the Resin.

This extract was a black, brittle solid, and amounted to 19 grams. It was digested with 500 c.c. of ether, when the greater portion passed into solution, while a small amount of a light green powder remained undissolved. The latter was collected, and boiled with alcohol, in which it was very sparingly soluble, in order to remove the green colouring matter. It was finally crystallised from a mixture of pyridine and alcohol, when it separated in colourless needles, melting and decomposing at about 290° . It yielded an acetyl derivative, melting at 166° , which was analysed:

0.0854 gave 0.2142 CO_2 and 0.0690 H_2O . $\text{C}=68.4$; $\text{H}=9.0$.
 $\text{C}_{21}\text{H}_{34}\text{O}_4(\text{CO}\cdot\text{CH}_3)_2$ requires $\text{C}=68.8$; $\text{H}=9.2$ per cent.

The above-described substance, when dissolved in chloroform with a little acetic anhydride, and a drop of concentrated sulphuric acid subsequently added, gave a pink coloration, rapidly changing to blue, and finally to green. It was evidently identical with the

dihydric alcohol designated as trifolianol, which had previously been isolated from the petroleum extract of the resin.

The above-mentioned ethereal solution of the ethereal extract was shaken successively with aqueous ammonium carbonate, sodium carbonate, and sodium hydroxide, and the various extracts thus obtained were separately examined. The ammonium carbonate removed only traces of a green resin. The first sodium carbonate extract yielded, on acidification, a dark green resin, which consisted chiefly of chlorophyll, whilst the subsequent sodium carbonate extract, when acidified, gave a nearly colourless precipitate. This was collected and dissolved in alcohol, when a small amount of a substance separated in colourless, talon-like needles, melting at 253° . This substance was found to be identical with the compound designated as pratol, $C_{16}H_{12}O_4$, which had previously been isolated from the portion of the alcoholic extract of clover flowers which was soluble in water. The mother liquors from the crystallisation of the pratol deposited a little of a crystalline substance, melting somewhat indefinitely between 178° and 183° , but the amount was too small to permit of its further examination. The final extraction of the ethereal liquid with sodium hydroxide yielded nothing but amorphous products, and on subsequently evaporating the ether only traces of a soft resin remained.

Chloroform and Ethyl Acetate Extracts of the Resin.

These extracts were black, brittle solids. They were small in amount, and nothing crystalline could be isolated from them.

Alcohol Extract of the Resin.

This was a dark brown, brittle solid, amounting to 43 grams. In order to ascertain whether it contained anything glucosidic, it was heated for some time with a 5 per cent. solution of sulphuric acid in aqueous alcohol, and the mixture subsequently distilled with steam, but no volatile oil or acid passed over. The distillation flask then contained, besides the aqueous liquid, a quantity of a black resin, which was collected and examined, but nothing definite could be isolated from it. The aqueous liquid, on the other hand, when extracted with ether, yielded a small quantity of a viscid, yellow oil, which gradually deposited a solid substance. The latter was crystallised from alcohol, when it separated in colourless needles, melting at 250° , and gave an acetyl derivative melting at 166° . It proved to be identical with the previously described substance, $C_{16}H_{12}O_4$, designated as pratol. The aqueous liquid,

which had been extracted with ether as above described, was freed from sulphuric acid by means of baryta, and concentrated to a small volume. It then readily reduced Fehling's solution, and yielded a small quantity of an osazone, melting at 203° . These facts indicated the presence of a glucoside in the alcoholic extract of the resin, and it is probable that the small amount of pratol, $C_{16}H_{12}O_4$, obtained from the latter represents one of its hydrolytic products.

Summary.

The detailed results of the present investigation of red clover flowers (*Trifolium pratense*, Linné) may be summarised as follows:

A quantity of the alcoholic extract, representing 37.1 kilograms of the dried flowers, when distilled with steam, yielded 10.5 grams, or 0.028 per cent., of essential oil. This essential oil, which contained furfuraldehyde, possessed the following constants: $d_{20^{\circ}/20^{\circ}} = 0.9476$; $\alpha_D + 4.0'$ in a 1-dcm. tube.

The portion of the alcoholic extract which was soluble in water contained a large amount of sugar, which yielded *d*-phenyl-glucosazone (m. p. 205°), but from the aqueous liquid the following definite substances were isolated: salicylic and *p*-coumaric acids; isorhamnetin, $C_{16}H_{12}O_7$ (m. p. 295°), which was probably present in the form of a glucoside; a number of new phenolic substances: pratol, $C_{15}H_8O_2(OH)(OCH_3)$, m. p. 253° , which apparently is a hydroxymethoxyflavone, and yields an *acetyl* derivative melting at 166° ; pratensol, $C_{17}H_9O_2(OH)_3$, m. p. 210° , which yields a *triacetyl* derivative melting at 189° ; a yellow compound, $C_{16}H_{10}O_7$ (m. p. 280°), which yields a *tetra-acetyl* derivative melting at $145-147^{\circ}$; a substance, $C_{15}H_7O_3(OH)_3$, m. p. 225° , which yields a *triacetyl* derivative melting at 209° ; a substance, $C_{14}H_{12}O_6$ (m. p. 214°); also the following new glucosides: trifolin, $C_{22}H_{22}O_{11}, H_2O$ (m. p. 260°), which yields on hydrolysis a yellow colouring matter, trifolitins, $C_{16}H_{10}O_6$ (m. p. 275°), and rhamnose, $C_6H_{12}O_5$; isotrifolin, $C_{22}H_{22}O_{11}$ (m. p. 250°), and a glucoside of quercetin, melting at 235° .

The portion of the alcoholic extract which was insoluble in water consisted chiefly of resinous material, the amount of which was equivalent to 5.6 per cent. of the weight of dried clover flowers. From the resin the following compounds were obtained: myricyl alcohol, $C_{31}H_{63}OH$; heptacosane, $C_{27}H_{56}$, and hentriacontane, $C_{31}H_{64}$; sitosterol, $C_{27}H_{46}O$ (m. p. $135-136^{\circ}$; $[\alpha]_D - 34.4^{\circ}$); a new dihydric alcohol, trifolianol, $C_{21}H_{34}O_2(OH)_2$ (m. p. 295°), which appears to be a homologue of ipuranol, $C_{23}H_{38}O_2(OH)_2$, and yields a *diacetyl* derivative melting at $165-166^{\circ}$ and having $[\alpha]_D - 25.7^{\circ}$; a mixture of fatty acids, consisting chiefly of palmitic, stearic, and

linolic acids, with small amounts of oleic, linolenic, and *isolinolenic* acids. A small amount of pratol, $C_{16}H_{12}O_4$, was likewise obtained, the latter having evidently been present in the resin in the form of a glucoside.

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