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INVESTIGATIONS ON CAESALPINIA BONDUCELLA

Part III.—Isolation of α-, β- and γ-Caesalpins and Determination of their Functional Groups

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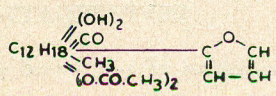
Extraction of the defatted kernel of *Caesalpinia bonducella* seeds with chloroform gave two crystalline bitter compounds named α-caesalpin, $C_{22}H_{32}O_8$, m.p. 187°C. and β-caesalpin, $C_{18}H_{26}O_5$, m.p. 243°C., and an amorphous bitter, m.p. 104-120°C. α-Caesalpin has been found to contain at least one hydroxyl group which is non-acetylable, two acetoxyl groups, one hindered carbonyl group and a furan ring. Alkaline hydrolysis of α-caesalpin gives β-caesalpin and acetic acid. β-Caesalpin contains at least two hydroxyl groups, only one of which is acetylable, one hindered carbonyl group and a furan ring. γ-Caesalpin on similar hydrolysis gives a crystalline bitter compound named as hydrolysed γ-caesalpin, m.p. 252°C., together with acetic acid and myristic acid. Hydrolysed γ-caesalpin contains at least three hydroxyl groups, two of which are acetylable, one potential carbonyl group and one furan ring. The presence of these functional groups has been demonstrated by chemical as well as physical methods such as ultraviolet and infrared absorption spectral analysis.

In some previous communications[1,2] we reported our findings on the bitter constituents of the seeds of *Caesalpinia bonducella* in the form of a short note in which we mentioned the availability, properties, medicinal uses etc. of the plant and also summarised all previous attempts to isolate and investigate the seed bitters. The present paper gives information, so far obtained, about the chemical and physical properties of these crystalline bitter constituents.

The kernel of mature seeds after removal of the fixed oil with light petroleum (b.p. 40-60°C.) on further extraction with chloroform at room temperature gave a non-crystalline solid bitter product in 0.92% yield. This bitter on cooling in ether solution afforded an ether-insoluble crystalline bitter compound, m.p. 140-148°C., and an ether soluble amorphous bitter substance, m.p. 104-120°C. Fractional crystallisation of the former from methanol gave two nicely crystalline, extremely bitter compounds—one as white rectangular plates, m.p. 187°C. (0.01%) and the other as colourless hexagonal plates (0.003%), m.p. 243°C. (decomp.) which we have called α-caesalpin and β-caesalpin, respectively. The amorphous bitter substance (0.73%) could neither be crystallised nor could its m.p. be improved by the usual methods of chromatography through alumina and we have called this amorphous substance γ-caesalpin.

α-Caesalpin is free from nitrogen, sulphur and halogen. It has a molecular formula of $C_{22}H_{32}O_8$. It is not a glycoside for it does not undergo acid hydrolysis giving reducing sugar. It does not dissolve in hot aqueous alkali nor does it evolve carbon dioxide with a bicarbonate, showing that it does not contain any lactonic or carboxylic group. It, however, gives strongly positive hydroxamic acid test providing an indication that it has ester group or groups. Infrared absorption spectrum of this compound (Fig. 1) has shown an ester carbonyl band[3] at 1733 $cm.^{-1}$ and a strong acetate band[3] at 1256 $cm.^{-1}$ affording strong evidence for the presence of ester groups corresponding to acetyl group. This has been confirmed by the alkaline hydrolysis of this compound with boiling aqueous alcoholic sodium hydroxide when another highly crystalline alkali-insoluble bitter compound m.p. 243°C. (decomp.) was obtained as colourless

hexagonal plates together with acetic acid from the alkaline solution after acidification with dilute sulphuric acid followed by solvent extraction. Acetyl determination of α-caesalpin has shown that it contains two O-acetyl groups. α-Caesalpin could not be acetylated by the usual methods of acetylation, although its infrared spectrum had shown a strong sharp characteristic hydroxyl peak[4] at 3572 cm.$^{-1}$ showing that it contains a hindered hydroxyl group (tertiary). There also has appeared another small but sharp peak at 3448 cm.$^{-1}$ in the hydroxyl region[4] of its infrared spectrum which indicates the possibility of another hindered hydroxyl group. Although intermolecular or intramolecular hydrogen bonding could well account for this peak, it is unlikely that this is so, since the hydroxyl groups which would obviously be required for this purpose are not unhindered and therefore they would not give rise to such effects. The infrared spectrum of α-caesalpin has also shown a carbonyl peak at 1714 cm.$^{-1}$ but it does not give semicarbazone or oxime under the usual conditions, nor does it give any precipitate with cold Brady's reagent.[5] When, however, boiled with a solution of 2,4-dinitrophenylhydrazine hydrochloride in alcohol, it gives a 2,4-dinitrophenylhydrazone as red nodules, m.p. 200°C. (decomp.). These facts prove that α-caesalpin has a hindered carbonyl group. Chemical tests such as colour reactions with *p*-dimethylaminobenzaldehyde and concentrated hydrochloric acid,[6] with Shear's reagent[7] and with acetic anhydride and concentrated sulphuric acid and also its sensitivity towards mineral acids turning it into gummy mass, strongly indicate that it contains a furan ring. This has further been confirmed by its ultraviolet and infrared absorption spectra. The ultraviolet spectrum shows peaks at 288 mμ (ε, 27.4) characteristic of β, γ-unsaturated ketonic group[8] and at 216 mμ (ε, 7801) characteristic of furan ring.[6] The infrared spectrum also shows clear characteristic peaks for furan ring[9] at 1506 and 873 cm.$^{-1}$ α-Caesalpin gave rise to peaks at 1431 and 1381 cm.$^{-1}$ in I.R. spectrum (KBr), characteristic of C-CH_3 group.[4] Thus all the oxygen atoms of α-caesalpin are practically accounted for. From these observations the formula of α-caesalpin may tentatively be put as:



The crystalline compound, m.p. 243°C. obtained above from α-caesalpin gives negative hydroxamic acid test as expected. This compound is insoluble in ether, petroleum ether, chloroform and benzene even on boiling, but is soluble in rather large volume of boiling methanol or ethanol. It is optically active, the specific rotation being $[\alpha]_D^{23} = +68.75°$. It has a molecular formula $C_{18}H_{26}O_5$. This formula suggests that the compound has been formed from α-caesalpin by normal hydrolysis of one of its acetoxyl groups and the elimination of its other acetoxyl group in the form of acetic acid under the influence of the alcoholic alkali used. This has further been confirmed by acetylation of this compound when a crystalline monoacetate was obtained in the form of needles (from benzene), m.p. 218°C., the molecular formula being $C_{20}H_{28}O_6$ corresponding to the above formula of the parent compound. The acetyl determination of this acetate has

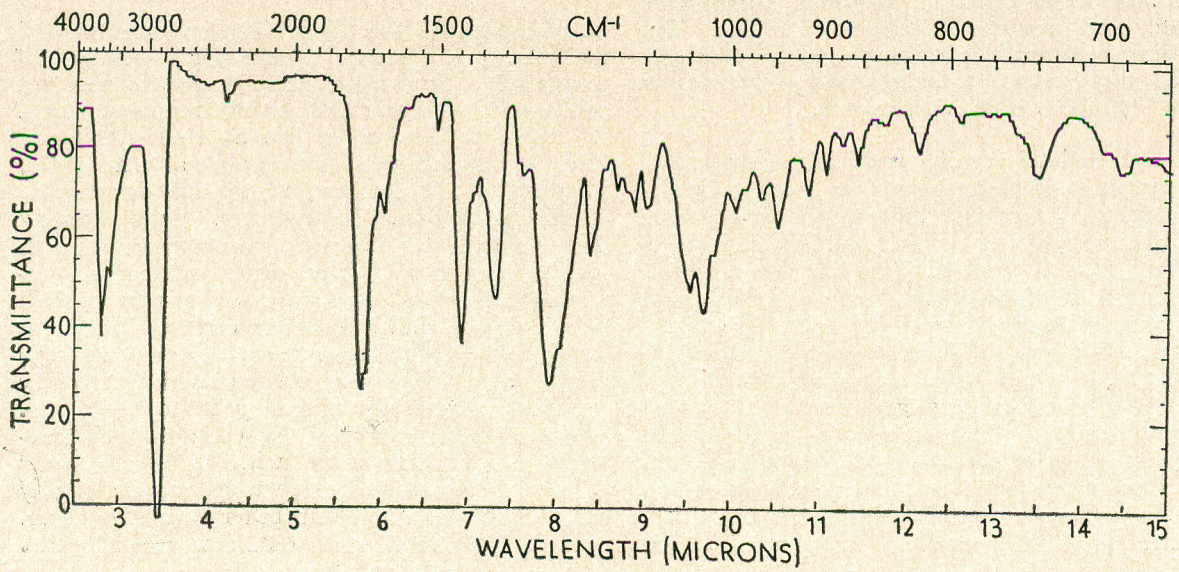


Fig. 1.—Infrared absorption spectra of α-caesalpin, $C_{22}H_{32}O_8$, m. p. 187°C.

shown 1.02, i.e. one acetyl group, and it also gives strong hydroxamic acid test, whence it may be concluded that the second acetoxyl group on being eliminated gave rise to a hydroxyl group that was not easily acetylated to give a diacetyl compound again. As expected, the compound, m.p. 243°C., has been found to contain hindered carbonyl group as is shown by its reaction with 2,4-dinitrophenylhydrazine reagent yielding only under drastic conditions a 2,4-dinitrophenylhydrazone as orange-red needles, m.p. 278°C. (decomp.) and by its non-reactivity towards the other carbonyl reagents. It also gives the characteristic colour reactions for furan ring.[6,7] The ultraviolet absorption spectrum of this compound shows peaks at 290 mμ (ε,27.4) and 216 mμ (ε, 8788), the former presumably being due to β, γ-unsaturated ketonic group[8] and the latter due to furan ring.[6] The infrared absorption spectra of this compound (Fig. 2) and of its acetate (Fig. 3) show peaks characteristic of all the expected functional groups. β-Caesalpin gave peaks at 1475 and 1389 cm.$^{-1}$ in I.R. spectrum (KBr) characteristic of C-CH_3 group.[4] Thus while the infrared spectrum of the former shows hydroxyl bands[4] at 3542 and 3279 cm.$^{-1}$, hinder-

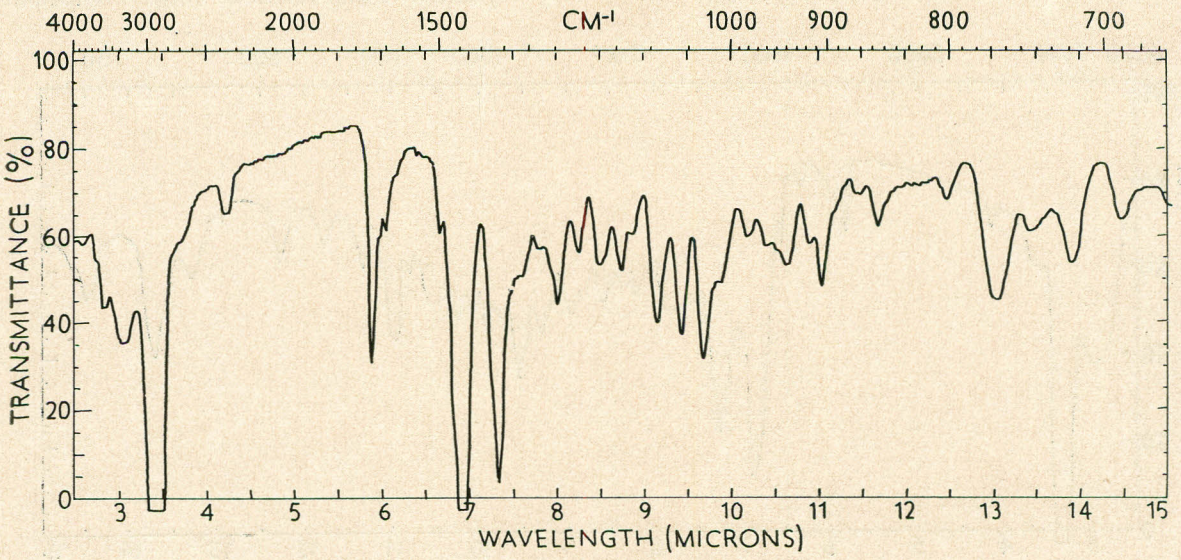


Fig. 2.—Infrared absorption spectra of β-caesalpin, $C_{18}H_{26}O_5$, m.p. 243°C.

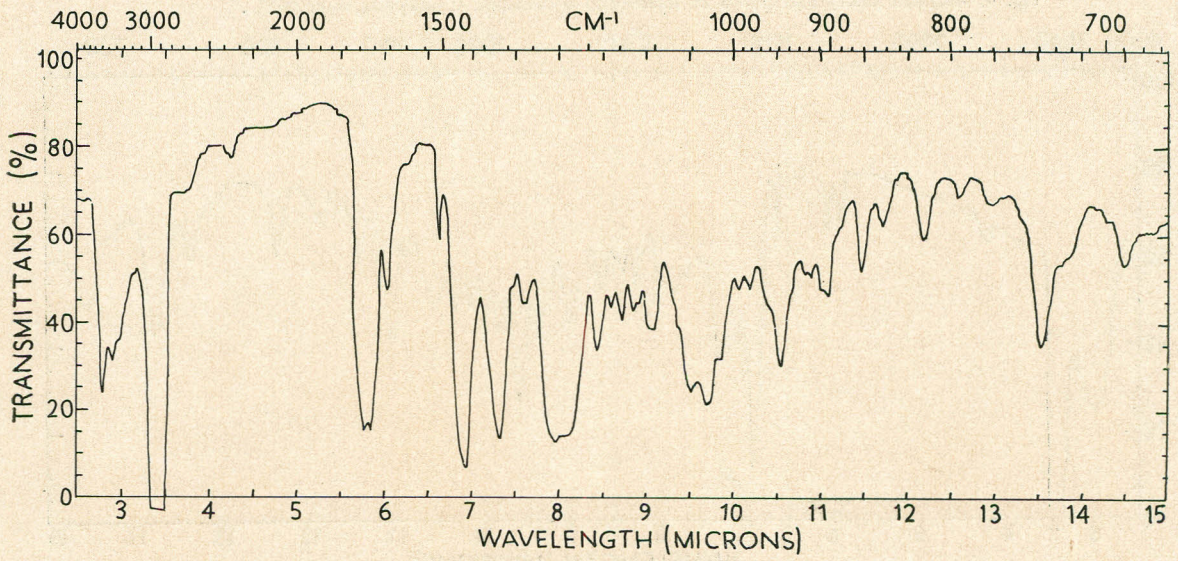


Fig. 3.—Infrared absorption spectra of β-caesalpin acetate, $C_{20}H_{28}O_6$, m.p. 218°C.

ed carbonyl band[4,10] at 1700 cm.$^{-1}$, furan ring peaks[9,10] at 1502 and 872 cm.$^{-1}$ having no acetate peak or ester carbonyl peak,[3] that of the latter has given non-acetylable hydroxyl peaks[4] at 3623 and 3497 cm.$^{-1}$, ordinary carbonyl peak[4] at 1712 cm.$^{-1}$, acetate carbonyl peak[3,10] at 1740 cm.$^{-1}$, acetate peak[3,10] at 1256 cm.$^{-1}$ and furan ring peaks[9,10] at 1508 and 872 cm.$^{-1}$.

β-Caesalpin has been found to be identical with the above compound, m.p. 243°C. This identity has been proved by their undepressed mixed m.p., identical specific rotation, and identical ultraviolet absorption spectrum. The formation of this compound in the seeds may be explained on the basis of a combined effect of hydrolysis and elimination of the acetoxyl groups of α-caesalpin by some enzymic action.

γ-Caesalpin, the amorphous bitter, when heated with dilute hydrochloric acid does not undergo hydrolysis giving any reducing sugar, thereby showing that it is not a glycoside. It is highly soluble in ether, chloroform, benzene, ethanol and methanol but is insoluble in petroleum ether and water. Alkaline hydrolysis of this susbstance with aqueous alcoholic sodium hydroxide gave a very crystalline alkali-insoluble bitter compound

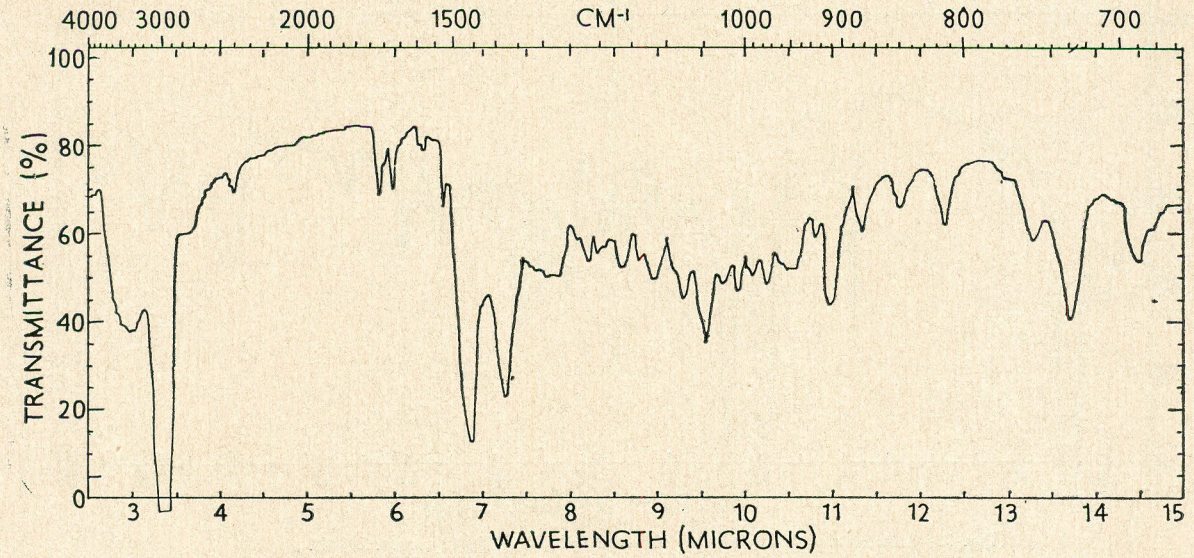


Fig. 4.—Infrared absorption spectra of hydrolysed γ-caesalpin, $C_{20}H_{30}O_6$, m.p. 252°C.

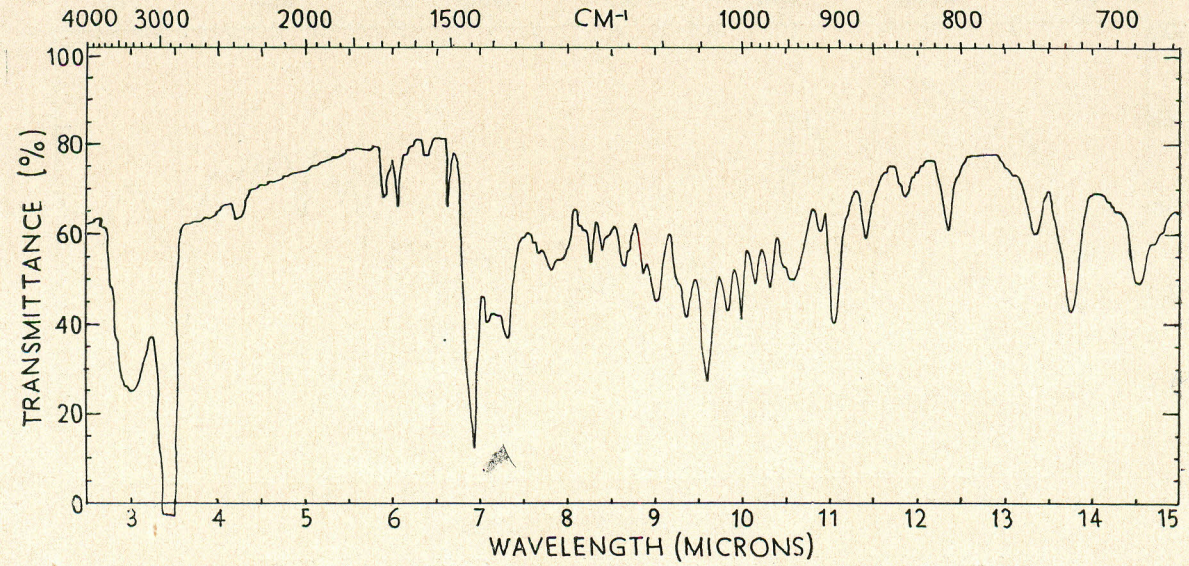


Fig. 5.—Infrared absorption spectra of hydrolysed γ-caesalpin, $C_{20}H_{30}O_6$, m.p. 222-224°C., after drying at 56°C., under vacuum.

as colourless hexagonal bars (from ethanol), m.p. 252°C. (decomp.), together with an appreciable quantity of acetic acid and some myristic acid. This crystalline bitter compound has been called hydrolysed γ-caesalpin which is insoluble in ether, petroleum ether, chloroform and benzene but moderately soluble in methanol and ethanol. γ-Caesalpin may thus be an ester of hydrolysed γ-caesalpin with acetic acid and myristic acid. Hydrolysed γ-caesalpin has a peculiar property of melting at different temperatures depending upon the solvent used for crystallisation.

When crystallised from dry ethanol, it gives hard colourless hexagonal bars, m.p. 252 °C., but, when crystallised from aqueous ethanol, it gives white fluffy needles, m.p. 222 °-224 °C. On some occasions its m.p. has also been found to lie in between 222 °-252 °C. This difference of m.p. is not due to any solvent of crystallisation as their m.ps. remained unchanged even when dried at ca. 118°C. at as low a pressure as 1 mm. of Hg for 8 hours. They are, in fact, one compound as shown by their identical combustion analyses and their infrared absorption spectra (Figs. 4, 5 and

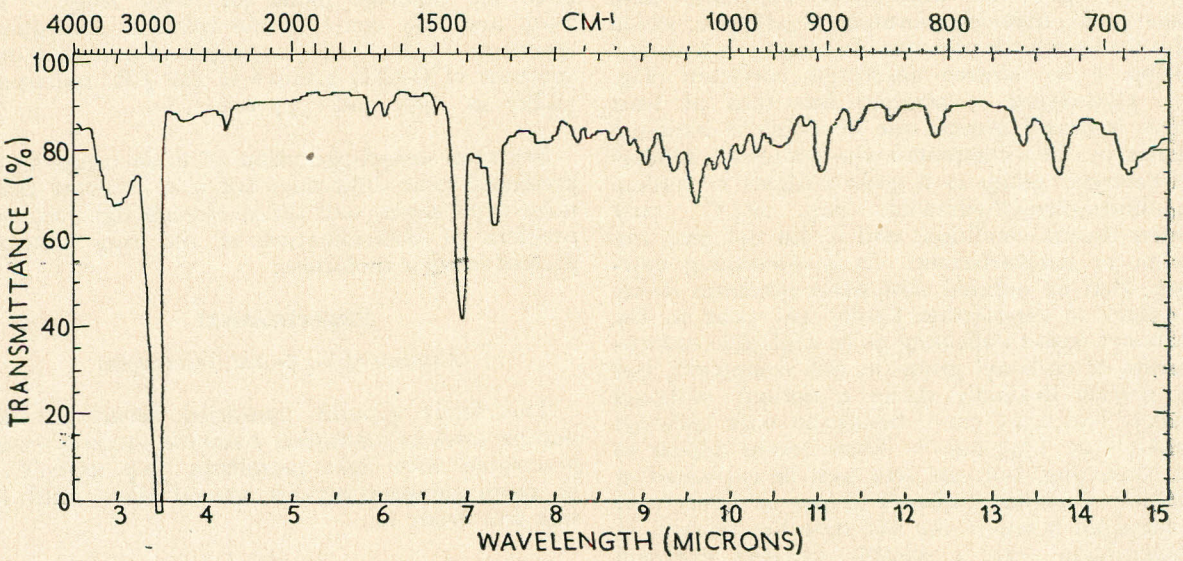


Fig. 6.—Infrared absorption spectra of hydrolysed γ-caesalpin, $C_{20}H_{30}O_6$, m.p. 222-224°C. after drying at 118°C. under vacuum..

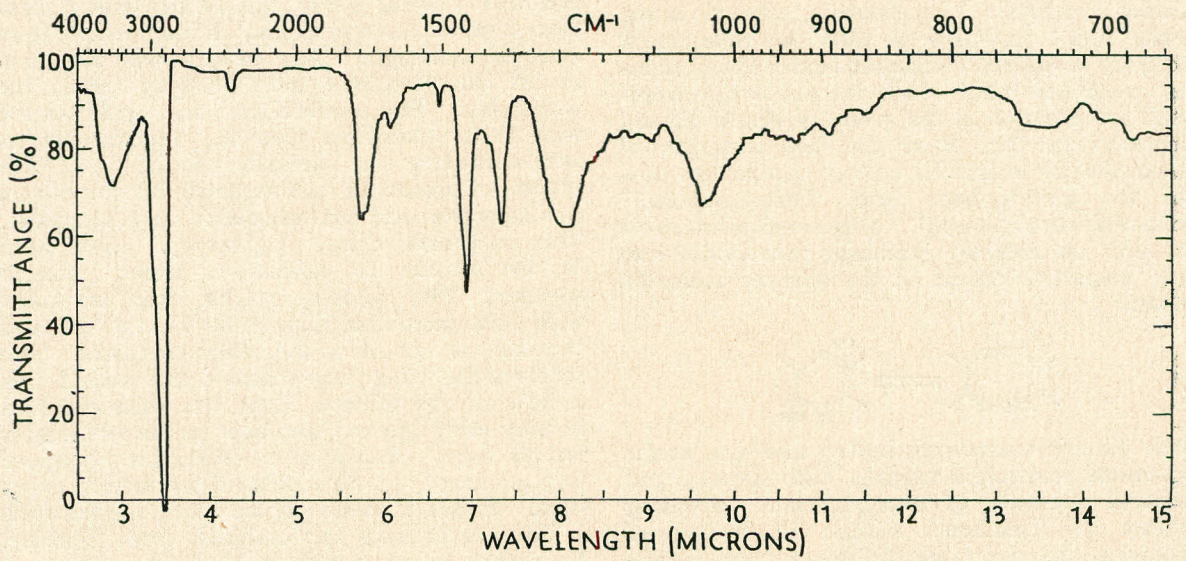
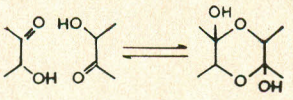


Fig. 7.—Infrared absorption spectra of diacetate of hydrolysed γ-caesalpin, $C_{24}H_{34}O_8$, m.p. 139-140°C.

6). We attribute this m.p. difference to the different crystalline shapes obtained from the different solvents used.

Hydrolysed γ-caesalpin is free from nitrogen, sulphur or halogen. It is neither an acid nor a lactone and it is free from any ester group as expected (negative hydroxamic acid test). It is optically active, the specific rotation being $[\alpha]_D^{30} = +40^\circ$. Its molecular formula is $C_{20}H_{30}O_6$. Its infrared spectrum shows characteristic peak at 3412-3300 cm.$^{-1}$ for hydroxyl group.[4] Acetylation of this compound with acetic anhydride and pyridine gave an amorphous diacetate, m.p. 139-140°C., molecular formula, $C_{24}H_{34}O_8$, which also has shown the presence of at least one hydroxyl group in its infrared absorption spectrum (Fig. 7). Hydrolysed γ-caesalpin has thus at least three hydroxyl groups, one of which is hindered (tertiary). This compound also contains a hindered carbonyl group as it gives a highly crystalline 2,4-dinitrophenylhydrazone, m.p. 264°C., only under drastic conditions and it did not give any oxime or semicarbazone. It is interesting, however, that its infrared absorption spectrum shows contrary to expectation hardly any peak in the carbonyl region indicating no or negligible concentration of carbonyl group in this compound, but its acetate distinctly shows a normal carbonyl peak[4,10] at 1721 cm.$^{-1}$ besides an ester carbonyl peak[3,10] at 1743 cm.$^{-1}$ These results appear to be conflicting, but can very well be explained as follows: that the carbonyl group in hydrolysed γ-caesalpin is not in the free state but remains in combination with a suitably situated hydroxyl group in this molecule forming an intramolecular hemiketal type of linkage, thus rendering its activity potential. The normal carbonyl group in the acetyl derivative is then the regenerated one from its parent compound under the acetylation conditions used. Such intramolecular hemiketals are common in the group of ketoses among carbohydrates, but there are also instances of intermolecular hemiketal type of compounds outside the carbohydrate field. Thus adipoin,[11] 3-oxo-*trans*-decalin-2α-ol,[3] 3-oxo-*cis*-decalin-2β-ol[3] etc. are, on keeping, gradually transformed into their respective dimers in the manner indicated below:



These dimeric compounds behave similarly as the hydrolysed γ-caesalpin towards both physical and chemical methods of analysis, thus providing support for hemiketal linkage in hydrolysed γ-caesalpin but the mode of formation of this linkage in hydrolysed γ-caesalpin is not intermolecular but intramolecular since the molecular size of its acetate fits in well with that of the compound, which otherwise would be different by analogy with these dimers.

Hydrolysed γ-caesalpin also contains furan ring as is shown by its characteristic colour reactions,[6,7] and it was sensitive towards mineral acids. In agreement with this observation this compound gave characteristic furan ring peaks at 1502 and 875 cm.$^{-1}$ in the infrared absorption spectrum[9,10] and at $\lambda_{max.}$ 215 mμ in the ultraviolet absorption spectrum.[6] Its acetate also gave the expected peaks for furan ring[9,10] at 1504 and 869 cm.$^{-1}$ in the infrared absorption spectrum. Hydrolysed γ-caesalpin also showed the presence of C-CH_3 group[4] in the I.R. spectrum (KBr) at 1453 and 1374 cm.$^{-1}$.

Selenium dehydrogenation of these compounds presented some difficulties but it is expected that these experiments will throw enough light on the problem of determination of the ring systems present in their molecules.

Experimental

Isolation of α-, β- and γ-Caesalpins

The finely ground, sun-dried kernel of the mature seeds of *Caesalpinia bonducella* (1000 g.) was percolated with light petroleum (b.p. 40-60°C.) at room temperature for several days until it was free from oil.

The defatted kernel thus obtained, after removal of the adhering petroleum ether by suction with a water pump, was soaked with chloroform (1500 cc.) in a percolator at room temperature and left overnight. The extract was then run out of the percolator drop by drop during the whole day. The residual mass was again treated with fresh chloroform (900 cc.) and the extraction operation was similarly carried out. This process of extraction was repeated till the extract was colourless and left no residue on evaporation. The combined extracts (4200 cc.) were freed of the solvent by distillation under reduced pressure. The viscous residue was triturated with light petroleum (b.p. 40-60°C., 150 cc.) and the mixture was left in the cooler overnight. The solid residue (9.2 g.) was collected and washed with a little of the solvent. This was then dissolved in pure ether (40 cc.) and kept in the refrigerator for 48 hours whereupon a crystalline substance (0.7 g.; 0.07% on the weight of the kernel) deposited. It was collected on the filter, washed with a little ether and recrystallised from benzene. It melted at 140-148°C. (not sharp) and gave negative tests for nitrogen, sulphur and halogen.

The substance was extremely bitter, and was readily soluble in methanol, ethanol, chloroform and acetic acid but was very sparingly soluble in ether and petroleum ether and was moderately soluble in benzene. This was fractionally crystallised from methanol to give on the one hand sharp, constant melting, extremely bitter, crystalline compound as white rectangular plates, m.p. 187°C. which we have named α-caesalpin and on the other hand another sharp melting, bitter compound was obtained as colourless hexagonal plates, m.p. 243°C., which we have called β-caesalpin.

The above ethereal mother liquor was evaporated to an amorphous solid residue (7.3 g.) which was highly soluble in chloroform, ether, benzene, methanol and ethanol but was insoluble in petroleum ether and water, and it had a m.p. 104-120°C. This amorphous bitter could not be crystallised from different solvents or mixed solvents. It was then chromatographed on alumina using different solvents such as benzene, chloroform, petroleum ether-chloroform as developing solvents but the m.ps. of the different fractions collected remained practically the same. We have called this product γ-caesalpin.

Tests on the General Properties of α-Caesalpin

Element tests of α-caesalpin according to Lassagne's method using sodium fusion technique showed that it did not contain nitrogen, sulphur or halogen. Its combustion analysis and molecular weight determination yielded the following results. Found: C, 62.53; H, 7.21; M.W.(Rast), 415. $C_{22}H_{32}O_8$ requires: C, 62.24; H, 7.60; M.W., 424. Its specific rotation measured in absolute alcohol (1% solution) was $[\alpha]^{23}_D = +37°$. This compound, when heated on the steam bath with dilute sulphuric or hydrochloric acid, gradually turned into a gummy mass. The aqueous portion of this reaction mixture on neutralisation with sodium carbonate solution was treated with Fehling's solution and boiled but there was no reduction, showing that the compound was not a glycoside. It was insoluble in aqueous sodium hydroxide solution and it did not react with saturated sodium carbonate or bicarbonate solution. Hydroxamic acid test was carried out with this compound according to the usual method and the test gave intense violet colour. Its infrared absorption spectrum in paraffin mull: $\lambda_{max.}$ 3572 (OH), 3448(OH), 1733 (ester carbonyl), 1714 (ordinary carbonyl), 1506 (furan ring), 1256 (acetoxyl group), 1190, 1150, 1122, 1106, 1048 (C-O stretching frequency in C-OH), 1031, 996, 966, 948, 917, 905, 873 (furan ring) cm.$^{-1}$ (full curve in Fig.1). It shows also a peak 1431 and 1381 cm.$^{-1}$, in I.R. spectrum (KBr) characteristic of C-CH_3 group. Ultraviolet and visible absorption spectra of this compound taken between the wavelengths 600-205 mμ in ethanol showed peaks at $\lambda_{max.}$ 216 mμ (ε,7801) and 288 mμ (ε,27.4). Colour tests of the compound were carried out as under for furan ring: (i) A solution of a small quantity of *p*-dimethylaminobenzaldehyde in about half cc. of concentrated hydrochloric acid was treated with only a few mg. of the compound when a pink colour developed quickly which is a positive test for furan ring. (ii) When treated with acetic anhydride and concentrated sulphuric acid,[7] it gave a greenish black colour. (iii) Treated with Shear's reagent in the cold,[7] it did not develop any colour but when warmed on the steam bath the mixture turned reddish brown characteristic of furan ring.

Alkaline Hydrolysis of α-Caesalpin

α-Caesalpin (2.9 g.) was dissolved in ethanol (20 cc.) by warming on the steam bath and a solution of sodium hydroxide (1 g.) in water (10 cc.) was added to it. The mixture was refluxed on the steam bath for 20 minutes and then most of the alcohol was removed under reduced pressure. The resulting alcoholic concentrate was cooled and diluted with water, whereby a substance in a nicely crystalline form began to separate. The mixture was left overnight and then the crystalline product was filtered, washed thoroughly with water and then dried in a desiccator over phosphorus pentoxide under vacuum. The product had a m.p. 243°C. and the yield was 1.85 g. Recrystallisation of this product from ethanol several times did not raise its m.p. It did not respond to hydroxamic acid test. An analytical sample made by recrystallisation of the compound from ethanol had a crystalline form of hexagonal plates, m.p. 243°C. (decomp.), $[\alpha]_D^{23} = +68.75°$. Found: C, 66.68; H, 7.67. M.W., (Rast), 317. $C_{18}H_{26}O_5$ requires; C, 67.03; H, 8.12; M.W., 322. It was extremely bitter to taste, and was soluble in boiling methanol or ethanol (a large volume of solvent was required) but insoluble in water, ether, petroleum ether, benzene or chloroform even when boiled with large excess of these solvents. Ultraviolet absorption spectra in ethanol gave peaks at $\lambda_{max.}$ 216 and 290 mμ (ε, 8788 and 27.4, respectively). Infrared absorption spectrum in paraffin mull: $\lambda_{max.}$ 3546 (OH), 3279 (OH), 1700 (ordinary carbonyl), 1648 (small break), 1501 (furan ring), 1091, 1058, 1031, 1011 (C-O stretching frequency in C-OH), 936, 907, 872 (furan ring) and 854 cm.$^{-1}$ (full curve in Fig. 2). It shows peaks at 1475 and 1389 cm.$^{-1}$ also indicating the presence of C-CH_3 group.

The alkaline filtrate obtained after separation of the above crystalline compound, m.p. 243°C., from the reaction mixture was continuously extracted with ether to remove any free organic matter. The aqueous solution was then acidified with 2N sulphuric acid, saturated with hydrated sodium sulphate and extracted with ether continuously for 36 hours. The ethereal extract was washed with a very small amount of water and then dried (Na_2SO_4). Removal of the solvent from this solution first on a water bath at 45-50°C. under atmospheric pressure followed by suction with the help of a water pump at room temperature afforded a liquid (0.7 g.) with a pungent smell like that of acetic acid. It was distilled to give a liquid, b.p. 105-115°C., highly soluble in water and strongly acidic to litmus paper; also when this liquid was treated with saturated sodium bicarbonate solution a vigorous effervescence took place. A portion of this liquid was neutralised with sodium carbonate in the usual way and was evaporated to a solid residue which gave intense red colour with ferric chloride solution and also gave positive cacodyl oxide test indicating that the liquid is acetic acid; it also gave characteristic odour of ethyl acetate when heated with ethyl alcohol and concentrated sulphuric acid. The remaining portion of the liquid was converted into the acid chloride with phosphorus trichloride, which was then made to react with an ethereal solution of *p*-toluidine and then the solution washed with dilute hydrochloric acid and water and dried over anhydrous Na_2SO_4. Removal of the solvent from this solution gave a crystalline residue, m.p. 140-142°C., which when recrystallised from ether-light petroleum (b.p. 40-60°C.) melted sharply at 147°C. Its mixed m.p. with an authentic sample of *p*-toluidide (prepared in the laboratory using pure acetic acid), m.p. 147°C., was undepressed. Thus the liquid was fully identified as acetic acid.

Determination of Acetyl Value of α-Caesalpin

α-Caesalpin (0.0827 g.) was mixed with exactly 10 cc. of approximately 0.15 N ethanolic potassium hydroxide solution and about 2 cc. of water in a round-bottom quick-fit flask and the mixture was refluxed for one hour on the steam bath. The solution was then cooled to room temperature, diluted with water and then titrated with 0.103 N sulphuric acid using phenolphthalein as indicator, which required 10.375 cc. of this acid. A blank determination carried out exactly similarly but without the addition of α-caesalpin required 13.95 cc. of the acid. Therefore, the alkali consumed by the quantity of α-caesalpin used was 13.95 − 10.375 = 3.575 cc. of 0.103 N potassium hydroxide. From this the acetyl value has been calculated out as 249.8 corresponding to 1.89 acetyl groups in the compound.

Attempted Acetylation of α-Caesalpin

α-Caesalpin (0.0280 g.) was dissolved in dry pyridine (0.5 cc.) and the solution was mixed with acetic anhydride (0.4 g.) The mixture was then heated on the steam bath for about 10 minutes after which it was left at room temperature for 48 hours. The solution was next treated with crushed ice when a crystalline solid separated, which was filtered, washed thoroughly with water and then dried (yield 0.0224 g.). It had a m.p. 185-186°C. It was recrystallised from methanol when it melted at 187°C.; its mixed m.p. with the starting material was undepressed.

2,4-Dinitrophenylhydrazone of α-Caesalpin

(*a*) *Treatment with the Reagent at Room Temperature.*—A little of α-caesalpin was dissolved in 3 to 4 drops of methanol and a few drops of Brady's reagent[5] were added to it. No precipitate appeared even on standing for 24 hours, although the colour darkened very slowly.

(*b*) *Treatment with the Reagent at the Boiling Point.*—2,4-Dinitrophenylhydrazine (0.15 g.) was treated with concentrated hydrochloric acid (0.5 cc.) and the yellow hydrochloride was dissolved in about 8 cc. of 2 N hydrochloric acid and then diluted with ethanol (8 cc.). This solution was heated to boiling. α-Caesalpin (0.12 g.) was then dissolved separately in ethanol (6 cc.) and boiled. The two hot solutions were mixed quickly and the mixture was boiled quickly for about one minute when a precipitate appeared. It was rapidly cooled under running water with stirring, and the crystalline precipitate was collected on the filter, washed with dilute hydrochloric acid and then thoroughly with water and then air-dried. It was then crystallised from ethyl acetate several times when a constant melting product was obtained as red nodules, m.p. 200°C. A mixed m.p. of this compound with 2,4-dinitrophenylhydrazine was depressed to 170°C.

Attempts to Prepare Semicarbazone and Oxime of α-Caesalpin

(*a*) α-Caesalpin (0.1031 g.) in ethanol (4 cc.) was mixed with an ethanolic solution of semicarbazide acetate prepared from semicarbazide hydrochloride (0.0780 g. equivalent to three carbonyl groups) and anhydrous sodium acetate (0.13 g.) according to the procedure described by Linstead and Weedon.[12] The mixture was

heated on the steam bath for about 30 minutes and was then left at room temperature for one hour but no solid separated. The reaction mixture was then kept in the refrigerator for about 18 hours but still no solid appeared. Then it was diluted with water whereby a crystalline solid separated which was collected on the filter, washed with water and dried at room temperature under vacuum. This product (0.0947 g.) had a m.p. 184°C. Recrystallisation of this product from methanol gave a solid m.p. and mixed m.p. with α-caesalpin, 187°C. It gave negative test for nitrogen.

(*b*) α-Caesalpin (0.0825 g.) dissolved in ethanol (4 cc.) was added to an ethanolic solution of hydroxylamine acetate made from hydroxylamine hydrochloride (0.05 g.) and anhydrous sodium acetate (0.09 g.) according to the method of Linstead and Weedon.[12] The solution thus obtained was refluxed on the steam bath for one hour and then cooled. But no solid separated from the solution. It was, therefore, diluted with water whereupon a solid separated, which was collected, washed with water and then recrystallised from methanol to give back only the starting material (0.0653 g. after drying), m.p. and mixed m.p. 187°C. This product also gave negative test for nitrogen.

Acetate of the Compound, m.p. 243°C. obtained from α-Caesalpin

The compound, m.p. 243°C., (0.60 g.) was heated on the steam bath with a mixture of acetic anhydride (1.1 cc.) and dry pyridine (1.2 cc.) until the solid had dissolved. The heating was continued for another 20 minutes whereafter the reaction mixture was left at room temperature overnight. The excess acetic anhydride was then decomposed by the addition of crushed ice. An oil separated which on scratching for a while solidified into a crystalline mass. It was collected on the filter, washed with water and dried, giving 0.62 g. of a crude product which gave strongly positive hydroxamic acid test. It was recrystallised from benzene three times when it had a constant m.p. 218°C., the shape of the crystals being small needles. This compound was highly soluble in chloroform, methanol and ethanol even in the cold but it was insoluble in ether and petroleum ether and was sparingly soluble in benzene. An analytical sample was made by recrystallisation of the compound from benzene followed by drying under a vacuum of less than 1 mm. of Hg at the temperature of boiling ethanol for 4 hours. Found: C, 65.80; H, 7.586. $C_{20}H_{28}O_6$ requires C, 65.92; H, 7.745. Infrared absorption spectrum in paraffin mull: $\lambda_{max.}$ 3623 (OH), 3497 (OH), 1740 (ester carbonyl), 1712 (normal carbonyl), 1685, 1508 (furan ring), 1256 (acetoxyl), 1051,1031, 1014, 949, 904, 827 (furan ring) and 822 cm.$^{-1}$ (full curve in Fig. 3).

Acetyl determination of this acetate was carried out as follows: The compound (0.0655 g.) was treated with an accurately measured 10 cc. of approximately 0.15 N alcoholic potassium hydroxide solution and 2 cc. water in a similar manner as for α-caesalpin and the excess alkali similarly titrated with 0.103 N sulphuric acid, which required 12.118 cc. of the acid whereas a blank required 13.825 cc. Therefore, the compound consumed 1.707 cc. of 0.103 N potassium hydroxide, thus corresponding to an acetyl value 156.2, i.e. 1.92 acetyl groups.

Attempts to obtain Semicarbazone and Oxime of the Compound, m.p. 243°C.

The reactions were carried out in a similar manner as described for α-caesalpin with 0.1257 g. of the compound for semicarbazone and 0.1194 g. for oxime but in both the cases only the starting material was recovered in 0.09822 g. in the case of the former and 0.09673 g. in the case of the latter.

2,4-Dinitrophenylhydrazone of the Compound, m.p. 243°C.

Treatment of the compound with Brady's reagent as for α-caesalpin at room temperature did not give any precipitate, but the solution darkened during 24-hour standing. The reaction was, therefore, carried out at the boiling point of the solvent as follows: The compound (0.30 g.) was dissolved in ethanol (20 cc.) by boiling on the steam bath and the solution was mixed with a boiling solution of 2,4-dinitrophenylhydrazine hydrochloride (0.5 g. of 2,4-dinitrophenylhydrazine and 1 cc. concentrated hydrochloric acid) made by dissolving the hydrochloride in about 15 cc. of 2N hydrochloric acid by heating followed by dilution with ethanol (15 cc.). This hot mixture was quickly dipped into boiling water with constant stirring and the boiling of the solution was continued for about a minute (a longer period of boiling resulted in an extensive polymerisation of the compound) when a large quantity of a dark red precipitate appeared. The mixture was rapidly cooled under running tap water and the precipitate was collected, washed with dilute hydrochloric acid and then thoroughly with water. The dark coloured precipitate (ca. 0.33 g.) m.p. 230-236°C. washed several times with 15 cc. portions of boiling ethanol (in which the DNP

was found to be very sparingly soluble) whereby a light yellow powder, m.p. 266°C. practically insoluble in ethanol remained. It was insoluble in chloroform, ethyl acetate, benzene, ether, petroleum ether, methanol and ethanol but was soluble in nitrobenzene and crystallised from a mixture of nitrobenzene and ethanol in fine orange red needles, m.p. 278°C. (decomp.).

Tests on the Properties of β-Caesalpin

β-Caesalpin did not give any test for nitrogen sulphur or halogen. Its mixed m.p. with the compound, m.p. 243°C. mentioned above, was undepressed. In the ultraviolet absorption spectrum it gave peaks at $\lambda_{max.}$ 216 and 290 mμ identical with those of the bitter m.p. 243°C. from α-caesalpin. Its optical rotation was $[\alpha]_D^{23} = +68.20°$. It is acetylated as follows: Treatment of β-caesalpin (0.032 g.) with acetic anhydride (ca. 0.2 cc.) and pyridine (0.2 cc.) first at ca. 100°C. for 15 minutes and then at 0°C. for about 30 hours followed by dilution with crushed ice gave an oil which soon solidified into a crystalline mass which was collected, washed with water and then recrystallised from benzene when it melted at 218°C. mixed m.p. with the acetate of the compound, m.p. 243°C., from α-caesalpin was undepressed.

Alkaline Hydrolysis of γ-Caesalpin, the Amorphous Bitter

A solution of γ-caesalpin (21.59 g.) in rectified spirit (100 cc.) was mixed with a 10% solution of sodium hydroxide (100 cc.) and the mixture was boiled under reflux on the steam bath for 20 minutes. Most of the alcohol was then distilled off under reduced pressure and the residual solution was allowed to cool to room temperature. A large crop of crystals in the shape of small needles separated which were collected on the filter, washed with a 1:1 mixture of ethanol and water. A clean and colourless crystalline solid was thus obtained which was first air-dried and then dried in a vacuum desiccator over P_2O_5; the yield was 4.27 g. It was crystallised several times from dry ethanol to give a constant melting compound in colourless hexagonal bars, m.p. 252°C. (decomp.), the yield being 3.48 g. We call this compound hydrolysed γ-caesalpin. It was extremely bitter and was insoluble in water, ether, petroleum ether, chloroform and benzene, and was moderately soluble in methanol and ethanol. Its mixed m.p. with β-caesalpin was depressed to 238°C. Sodium fusion test did not show the presence of nitrogen, sulphur and halogen. An analytical sample made by recrystallisation of this sample eight times from dry ethanol followed by drying at 1 mm. pressure and at 120°C. had a m.p. 252°C. and afforded the following analytical results. Found: C, 65.64; H, 8.24; M.W.(Rast), 362. $C_{20}H_{30}O_6$ requires C, 65.55; H, 8.25; M.W., 366.44. $[\alpha]_D^{30} = +40°$. Infrared absorption spectrum in paraffin mull: $\lambda_{max.}$ a broad band at 3412-3300 $cm.^{-1}$ (OH), a very small peak at 1702 $cm.^{-1}$ (indicate negligible concentration of ordinary carbonyl), 1502 (furan ring), 1055, 1015, 1003, 906, 875 (furan ring), 843 $cm.^{-1}$ (full curve in Fig. 4). Hydrolysed γ-caesalpin also shows peaks at 1453 and 1374 $cm.^{-1}$ in KBr indicating the presence of C-CH_3 groups. Ultraviolet absorption spectrum in ethanol: $\lambda_{max.}$ 215 mμ (ε8427). This compound melting at 252°C. when dissolved in ethanol and the ethanolic solution concentrated to a small volume and then mixed with 2 to 3 times its volume of water crystallised in rather fluffy needles which had m.p. 222-224°C. Its mixed m.p. with the original substance was ca. 237°C. It was dried in a drying pistol by heating at the temperature of boiling acetone under vacuum when also it had the same m.p. i.e. 222-224°C. Found: C, 65.69; H, 8.25; M.W. (Rast), 365. Its infrared spectrum was identical with that of the above sample, m.p. 252°C. (full curve in Fig. 5). In order to further ascertain whether this lowering of m.p. was due to water of crystallisation, this sample was dried under vacuum (1 mm. pressure of Hg) at the temperature of boiling *n*-butyl alcohol for 8 hours but its m.p. was still the same, i.e., 222-224°C., and it gave the following analytical results: Found: C, 65.51; H, 8.17. Its infrared spectrum was exactly identical with the above (full curve in Fig. 6). When this lower melting substance was recrystallised again from pure ethanol, crystals in the form of hexagonal bar, m.p. 252°C. (decomp.) were obtained.

The alkaline solution left after removal of the hydrolysed γ-caesalpin from the hydrolysis mixture of γ-caesalpin was continuously extracted with ether to remove any basic or neutral organic matter. Three such batches of ether-extracted alkaline aqueous solutions were combined together and then acidified with 5 N sulphuric acid whereby a dark coloured oil came out. The oil was separated, dissolved in ether and washed successively with dilute sulphuric acid and water and then dried over anhydrous sodium sulphate. Removal of the solvent from the solution then afforded a viscous dark brown mass (ca. 15 g.), which was separated by Twitchel's lead salt method[13] into a saturated fatty acid (0.94 g.), m.p. 54°C. (after several recrystallisations from aqueous alcohol). This was myristic acid as confirmed by preparing its anilide, m.p. 84°C.,

according to the method given in the literature[14] and a resinous gum. The aqueous acidic solution after removal of the oily product was saturated with hydrated sodium sulphate and then continuously extracted with ether for 36 hours. The ethereal extract was dried over anhydrous sodium sulphate and the ether then removed at a low temperature (40-45 °C. bath temperature under atmospheric pressure) when a liquid residue (ca. 10.25 g.) was obtained, which was distilled under atmospheric pressure using a small Vigraux fractionating column. About 0.4 g. of the liquid distilled at 35-55°C. which was mostly ether as it gradually evaporated on standing leaving no residue, about 1 g. distilled at 55-105°C. and about 7 g. distilled at 105-114°C. The last fraction was redistilled to give a liquid, b.p. 115-117°C. This liquid responded to all the tests for acetic acid which was confirmed by the preparation of acet-*p*-toluidide from this liquid according to the procedure used for the acetic acid obtained from α-caesalpin described before.

Diacetate of Hydrolysed γ-Caesalpin

Hydrolysed γ-caesalpin (1.75 g.), acetic anhydride (5 cc.) and dry pyridine (6 cc.) were mixed together and the mixture was heated on the steam bath for 30 minutes and then left at room temperature for 24 hours. The solvent was then removed at room temperature under reduced pressure and the viscous residue thus obtained was diluted with about 3 cc. of ice cold water. The separated viscous mass was scratched with the help of a spatula with cooling in ice whereby the mass became an amorphous solid. The solid was collected on the filter, washed thoroughly with water and then dried in a vacuum desiccator over P_2O_5 to give 1.80 g. of a solid, m.p. 139-140°C. It was highly soluble in ethanol, methanol, ether, benzene and chloroform even in the cold but was insoluble in petroleum ether and water. All attempts to crystallise it using solvents and mixed solvents failed. The amorphous substance was then dissolved in ca. 3 cc. of methanol and then filtered. Careful removal of the solvent from the filtrate at room temperature under a reduced pressure, followed by drying under vacuum in a drying pistol at the temperature of boiling ethanol, gave the analytical sample of the diacetate, m.p. 139-140°C. Found: C, 64.253 ; H, 8.03 ; M.W. (Rast), 448. $C_{24}H_{34}O_8$ requires C, 63.97; H, 7.61; M.W. 450. Infrared absorption spectrum in paraffin mull: $\lambda_{max.}$ 3520 (OH), 1743 (ester carbonyl), 1721 (ordinary carbonyl), 1504 (furan ring), a broad band between 1250-1221 (acetoxyl), 1045, 902, 869 (furan ring) $cm.^{-1}$ (full curve in Fig. 7). The compound gave strongly positive hydroxamic acid test.

The acetyl determination of the above acetate was carried out as follows: The compound (0.1730 g.) was mixed with accurately measured 10 cc. of approximately 0.15 N alcoholic potassium hydroxide solution and 2 cc. of water. The mixture was refluxed on the steam bath for 1 hour, cooled to room temperature, diluted with water, and the excess alkali titrated with 0.103 N sulphuric acid using phenolphthalein as indicator. 6.775 cc. of the acid was consumed in this titration. A blank determination was carried out under exactly similar conditions which took up 13.975 cc. of the acid. Therefore, the consumption of 0.103 N potassium hydroxide by the above quantity of the acetate was 7.20 cc. which corresponds to an acetyl value of 240.5, i.e. 1.93 acetyl groups.

Chemical Tests for Carbonyl Group in Hydrolysed γ-Caesalpin

Attempts were made to prepare semicarbazone or oxime of hydrolysed γ-caesalpin by a similar method as described for α-caesalpin. But no semicarbazone or oxime could be obtained; only the starting material was recovered in almost quantitative yield. The compound also did not react with cold Brady's reagent. However, when treated with boiling 2,4-dinitrophenylhydrazine hydrochloride, it gave crystalline DNP as follows:

Hydrolysed γ-caesalpin (0.7 g.) dissolved in 30 cc. of boiling ethanol was treated with 2,4-dinitrophenylhydrazine hydrochloride reagent (prepared from 1 g. 2,4-dinitrophenylhydrazine and 2 cc. of concentrated hydrochloric acid followed by dissolving the salt in 20 cc. 2 N hydrochloric acid diluting it with 20 cc. ethanol) similarly as α- and β-caesalpins. The dark brown precipitate formed was collected on the filter, washed with dilute hydrochloric acid and then thoroughly with water. This was followed by washing a number of times with boiling ethanol and boiling ethyl acetate (in both these solvents the DNP was almost insoluble) whereby a light yellow precipitate (0.38 g.) m.p. 261°C. (decomp.) was obtained. This was crystallised from a mixture of nitrobenzene and ethanol to give the 2,4-dinitrophenylhydrazone in beautiful orange needles, m.p. 264°C. (decomp.). (The low yield was due to extensive polymerisation of the acid sensitive compound during the reaction).

Colour Tests for Furan Ring of Hydrolysed γ-Caesalpin

The colour reactions were carried out in a similar manner as described for α-caesalpin and exactly similar results were obtained.

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