INVESTIGATIONS ON CAESALPINIA BONDUCELLA

Part II.—Chemical Examination of the Leaves

M. Qudrat-i-Khuda, M. Erfan Ali and Quazi Anwaruddin Ahmed

Natural Products Research Division, East Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Dacca

(Received July 1, 1961)

Chloroform extraction of the leaves gave a white amorphous bitter principle, $C_{24}H_{32}O_8$, m.p. 119-120°C. in 0.035% yield and a waxy material. Infrared spectra of the bitter shows that it contains hydroxyl and acetoxyl groups and also probably furan ring. The presence of furan ring is further indicated by colour reactions. Saponification of the wax has given myristic acid and a non-crystalline alcohol. The debittered and dewaxed leaves on alcohol extraction gave a highly crystalline sweet product, m.p. 187°C., in about 4% yield ; this compound has been characterised as pinitol. A small proportion of a reducing sugar characterised as glucose has also been detected in the leaves.

Introduction

As described in previous communications[1,2] *Caesalpinia bonducella* is a wild medicinal plant abundantly available in East Paskistan. The entire plant is used medicinally. The constituents of seeds have been described in Part I of this series of papers. The leaves of this plant are also bitter and they have been in use as an antimalarial for a long time although to a limited extent. A survey of literature has shown that no attempts have ever been made so far to examine the bitter or other constituents of these leaves. The success achieved in these laboratories in isolating crystalline bitter principles from the seeds stimulated our interest to examine the leaves as well.

The dry mature leaves were first extracted with chloroform at room temperature. The extract after removal of the chlorophyll and solvent gave a petroleum ether insoluble amorphous greyish white bitter substance, m.p. 46-65°C., and a petroleum ether soluble bitterless waxy material. The bitter substance on being chromatographed through alumina column afforded in poor yield a pure white amorphous bitter melting sharply and constantly at 119-120°C. This compound has a molecular formula, $C_{24}H_{32}O_8$. It is highly soluble in common organic solvents but it does not dissolve in petroleum ether and water and all attempts to crystallise it failed. It is optically inactive and is neither an acid nor a lactone as it does not dissolve in caustic alkali even when heated. It is not a glycoside either, since its acid hydrolysis does not afford any reducing sugar. It, however, gives a positive hydroxamic acid test suggesting it to be an ester. The ester character of the compound must be at least partly due to the acetyl group, the presence of which has been strongly indicated by the appearance of a prominent characteristic peak at 1233 cm.$^{-1}$ in the infrared absorption spectra of this compound,[3] (full curve in Fig. 1) which also indicates the presence of hydroxyl group[4] in it at 3596-3460 cm.$^{-1}$. The peak at 1749 cm.$^{-1}$ is due to ester carbonyl,[3] presumably the acetyl carbonyl. Although colour reactions of this compound such as its reaction with *p*-dimethylaminobenzaldehyde and concentrated hydrochloric acid,[1] with Shear's reagent[5] and reaction with acetic anhydride and concentrated

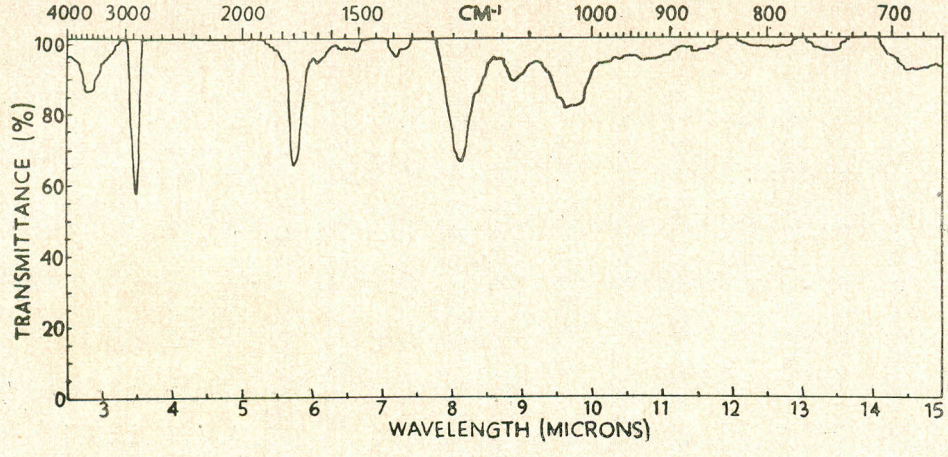

Fig. 1.—Infrared absorption spectra of the white amorphous bitter principle, $C_{24}H_{32}O_8$, m.p. 119—120°C.

sulphuric acid[5] all give positive indications for the presence of furan ring, the physical methods do not fully confirm it. Thus while the two expected peaks for furan rings[6] at around 1505 and 875 cm.$^{-1}$ in infrared spectra only appeared as very weak peaks, the expected bank[1,7] at ca. 215 mμ in the ultraviolet spectra was completely absent. Further investigations are in progress.

The waxy material on saponification gave an acid and an alcohol. The acid is a crystalline compound, m.p. 54°C., which gives an anilide, m.p. 83-84°C., and has an equivalent weight, 227.7. The acid has thus all the properties and characteristics of myristic acid[8] and is, therefore, identical with it. The alcohol part could not be obtained in crystalline form and is still under investigation.

The chloroform extracted leaves were next percolated with rectified spirit and the percolate freed from chlorophyll as usual. The product thus obtained was a mixture consisting mainly of a highly crystalline non-reducing sweet product and a small proportion of a reducing syrupy mass.

The sweet crystalline compound has a m.p. 187°C., and is highly soluble in water, sparingly soluble in methanol and ethanol and is insoluble in non-polar solvents. It is optically active having specific rotation of +66° in water. Although these properties correspond exactly to those of sucrose, this compound is not sucrose as its mixed m.p. with sucrose is depressed by about 30°C., and unlike sucrose it does not reduce Fehling's solution when hydrolysed with dilute hydrochloric acid. Further examination of this compound has shown that it has a molecular formula, $C_7H_{14}O_6$ and contains one methoxyl group. Althoug it did not give any crystalline acetyl derivative, a crystalline diisopropylidene derivative, m.p. 104°C., could easily be obtained. A thorough search of literature has revealed that pinitol [9,10,11] a monomethyl ether of *d*-inositol, has all these properties in common and, therfore, the compound under investigation is pinitol. We call this compound here 'caesalpinitol' to indicate its source.

The infrared absorption spectral curve of this compound is shown in Fig. 2. It shows a band at 3472-3320 cm.$^{-1}$ indicative of its hydroxyl groups[4] and a C-O-C band[12] at 1125 cm.$^{-1}$ confirming methoxyl group. As expected no other functional groups are indicated by this spectrum.

In view of the fact that caesalpinitol is a monomethyl ether of inositol, which is a growth promoting chemical, it will be of interest to examine its activity in this direction, and the results will be reported later.

The reducing substance has been found to be glucose as indicated by the formation of glucosazone and confirmed by paper chromatography. This reducing sugar is present in the leaves to the extent of 0.109 %.

Experimental

Isolation of the Bitter Priniciple of the Leaves of Caesalpinia bonducella.—Green mature leaves of *Caesalpinia bonducella*, collected in the month of July from the locality of Dacca, were semi-dried by keeping for a few days at room temperature. They were then powdered and extracted as follows: 400 g. of the powder was soaked with 2500 ml. of chloroform in a percolator at room temperature and left overnight. The deep-green extract was then run out drop by drop into a conical flask. Fresh chloroform (1500 ml.) was added to the powder in the percolator and the extraction was carried out similarly. This process of extraction was repeated four times. The combined extracts were passed through a column of charcoal (powdered

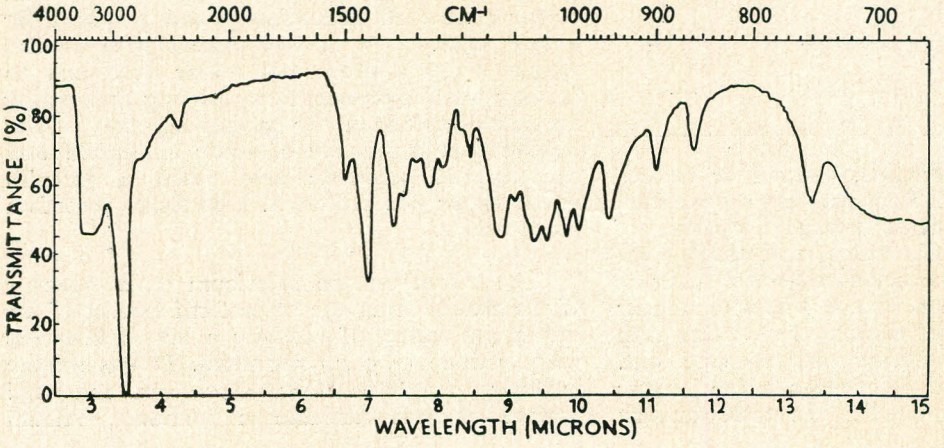

Fig. 2.—Infrared absorption spectra of the event crystalline compound, $C_7H_{14}O_6$, m.p. 187°C. (caesalpinitol).

gas absorbent charcoal) whereby an almost colourless solution was obtained. The minimum size of the column required for this purpose was 36 × 5 cm. The chlorophyll-free chloroform solution thus obtained was distilled under reduced pressure. The solvent was distilled off, leaving a brown viscous mass. Removal of last traces of the solvent from the residue followed by trituration with 100 ml. light petroleum (b.p. 60-80°C.) gave a greyish white precipitate, which was collected by filtration through a Buchner funnel and washed with some more solvent. Drying the precipitate under vacuum at room temperature afforded about 2 g. of an amorphous bitter solid, m.p. 46-65°C. It was highly soluble in ether, acetone, chloroform, benzene, methanol, ethanol and ethyl acetate but was insoluble in petroleum ether and water. It could not be obtained in crystalline form from solvents or mixed solvents. The whole mass was, therefore, dissolved in the minimum volume of chloroform and added on to the top of an alumina column (24 × 2 cm.). The chromatogram was then eluted with a mixture of 2:1 chloroform and light petroleum (b.p. 40-60°C.). The result of this chromatography is given in Table. 1.

The fractions separated were all bitter and amorphous. Thus it will be seen from the table that most of the original stuff was absorbed in the column, which, however, could not be eluted out

TABLE 1.

No. of fractions	Volume collected	Weight of residue after evaporation	m.p. of residue
1—5	5 × 10 ml.	nil	—
6	10 ml.	0.16 g.	90°C.
7	,,	0.24 g.	89°C.
8	,,	0.10 g.	87°C.
9	,,	nil	—

even with boiling methanol, ethanol or chloroform. The separated fractions were combined together and again passed through a column of alumina of the same dimension, and eluted with the same solvent the fractions collected being in portions of 5 ml. The substance began to appear in the 12th fraction and continued up to the 18th fraction after which no further substance came out. All the fractions were amorphous white bitter solids melting at 119-120°C., the total amount being 0.14 g. Further chromatography gave the same results. This compound was highly soluble in common organic solvents except petroleum ether and water, and it could not be crystallised. It was, therefore, dissolved in pure chloroform, filtered from the impurities, the solvent removed and the residue treated with pure light petroleum (b.p. 40-60°C.). The amorphous bitter solid thus obtained was dried by heating at the temperature of boiling acetone under a vacuum of less than 1 mm. of Hg and this was treated as a pure compound. Sodium fusion of this compound followed by subsequent tests of the product for the different elements showed that it did not contain nitrogen, sulphur or halogen. Its elemental analysis and molecular weight determination gave the following results: Found: C, 64.26; H, 7.21; m.w. (Rast) 453. $C_{24}H_{32}O_8$ requires C, 64.28; H, 7.19; m.w. 448.49. Its specific rotation was nil. The substance was insoluble in 10% aqueous sodium hydroxide solution even when boiled. When heated with dilute hydrochloric acid on the steam bath it gradually turned dark brown and then gummy; the filtrate from this acid treatment was neutralised with dilute sodium hydroxide solution and then heated with Fehling's solution but there was no reduction showing that the compound was not a glycoside. Hydroxamic acid test carried out according to the usual method gave an intense violet colour. Infrared absorption spectra of this compound in Nujol mull: $\nu_{max.}$ a shoulder 3596-3460 (OH), 2865, 1749 (ester carbonyl), a weak peak 1508 (furan ring?), 1233 (acetyl group) and a very weak peak 875 (furan ring?) cm.$^{-1}$. The full curve is given in Fig. 1. There was no peak in the ultraviolet absorption spectra between the wavelengths 210-350 mμ of this compound. The following colour reactions were carried out for furan rings in this compound: (a)[1,7] A few mg. of *p*-dimethylaminobenzaldehyde was dissolved in a small quantity of concentrated hydrochloric acid in a test tube and then mixed with a few mg. of the compound; a pink colour developed immediately and persisted for a long time. (b)[5] It was treated with Shear's reagent when a brown-red colour developed on heating which intensified on standing thus giving a positive indication for furan ring. (c)[5] It was treated with a mixture of acetic anhydride and concentrated sulphuric acid when a greenish violet colour was produced. Literature[5] records a green colour.

The solvent of the petroleum ether triturate of the residue from the chloroform extract of the leaves was taken off whereby a brown bitterless waxy residue (3.5 g.) remained. It was soluble in ether, petroleum ether, chloroform and ethyl acetate but was insoluble in methanol, ethanol

and water. Charcoaling did not remove the colour.

Hydrolysis of the Wax.—The wax (3 g.) was mixed with 10% alcoholic potassium hydroxide solution (20 ml.) and the mixture was refluxed on the steam bath for 3 hours. The alcohol was distilled off and the viscous residue was treated with 30 ml. water. The insoluble residue was extracted out continuously with ether. Drying (Na_2SO_4) of the ethereal extract followed by removal of the solvent gave a yellowish semi-solid substance (1.6 g.) which is readily soluble in ether, petroleum ether and chloroform but insoluble in cold methanol or ethanol. Hot methanol, however, dissolved the substance on cooling. Liebermann-Burchard test gave purple-violet colour. This is the alcohol part of the wax and could not be crystallised yet. Investigations on the compound are continuing.

The alkaline aqueous solution left after the ether extraction was acidified with dilute sulphuric acid whereby an oily mass separated, which was extracted out with ether exhaustively. The ethereal extract was washed with water and dried (Na_2SO_4). Removal of the solvent gave a dark brown semi-soild acidic product (0.45 g.). This acid was collected from several experiments and then the crude acid (1.69 g.) was separated into a solid saturated acid (0.23 g.), m.p. 52-54°C., and a brown resinous mass by the method of Twitchell.[13] The solid acid crystallised as white small needles from aqueous alcohol when it melted sharply at 54°C. Further crystallisation did not raise its m.p. Its equivalent weight determined according to the literature method[8] was 227.7 and it gave a crystalline anilide, m.p. 84°C., prepared according to the method of Ahmad and Hahn[14] and crystallised from methyl alcohol.

Isolation of Caesalpinitol from the Leaves.—The residual leaves after the chloroform extraction were percolated at room temperature with rectified spirit (2000 ml.) in a percolator and the mixture was left overnight. The extract was drained out. Two further extractions were similarly carried out each time with about 1000 ml. of the solvent. The combined light green extract was decolourised by passing it through a column of powdered gas absorbent charcoal (36 × 5 cm.). The clear practically colourless solution was then distilled under reduced pressure. The residue was freed from the last traces of solvent by suction with a vacuum pump at about 50°C., whereby a brown viscous syrupy mass (21 g.) was abtained. On standing for a few days at room temperature it was found that a lump of crystal had separated from this syrupy mass. The mass was then stirred with the help of a spatula whereupon the whole mass solidified with evolution of heat. This was dissolved in methanol by refluxing on the steam bath and filtered hot from the impurities. The brown methanolic solution was then seeded with a trace of the above solid and allowed to stand. Prismatic crystals separated, which were collected on the filter, washed with a little fresh solvent and then air-dried. The crystalline product thus obtained was a white substance (16.27 g.), m.p. 187°C. (sharp). Repeated crystallisation from the same solvent did not raise its m.p. It was highly soluble in water, moderately so in hot methanol and ethanol but was insoluble in ether, petroleum ether, chloroform, benzene and ethyl acetate. It was sweet to taste and did not reduce Fehlings' solution. Its specific rotation was $[\alpha]_D^{34}$ (in water) = + 66°. Its mixed m.p. with sucrose, m.p. 187°C., was 163°C. A little of this compound was heated with dilute hydrochloric acid on the steam bath for about 15 minutes, the acid was neutralised with sodium hydroxide solution and then boiled with Fehling's solution, but no reduction took place. Its combustion analysis and molecular weight determination gave the following results: Found: C, 43.49; H, 7.13; m.w. (cryoscopic method using water as solvent) 191.40. Calculated for $C_7H_{14}O_6$: C, 43.31; H, 7.23; m.w. 194.18. Infrared absorption spectra in Nujol mull: $\nu_{max.}$ a shoulder 3472-3320 cm.$^{-1}$ (OH),2880, 1437, 1363, 1125 (-OCH_3) cm.$^{-1}$, complex band between 1070-1000 cm.$^{-1}$, 964, 900, 860 cm.$^{-1}$ (Full curve in Fig. 2).

Qualitative detection of methoxyl group in this compound was carried out according to the method of Tobie[15] by generating methyl iodide by the method of Zeisel and reacting the iodide with mercuric nitrate solution. A prominent vermilion colour developed showing the presence of methoxyl group. Quantitative estimation of methoxyl group by the usual Zeisel's method showed 14.99% of methoxyl group in this compound corresponding to one methoxyl group for the formula $C_7H_{14}O_6$ which theoretically requires 14.76% for one methoxyl group.

Acetylation of this compound was carried out as follows:

(i) The compound (0.5 g.), fused sodium acetate (0.5 g.) and acetic anhydride (4 ml.) were mixed together and heated on the steam bath for 3½ hours. The homogeneous solution was then treated with crushed ice whereby a viscous liquid separated which did not solidify even on long scratching. It was, therefore, extracted with ether, the ethereal extract washed with sodium bicarbonate solution and water and then dried

(Na_2SO_4). Removal of the solvent gave a viscous liquid (0.45 g.) which could not be crystallised.

(ii) The acetylation was then carried out by the method of Griffin and Nelson[16] by warming the compound (0.5 g.) with acetyl bromide (2.2 g.) for about half an hour followed by decomposition of the excess bromide with crushed ice, extraction with ether, washing the ethereal extract successively with sodium bicarbonate solution and water, and then removal of the solvent from the dried (Na_2SO_4) extract. The residual product was a viscous mass which offered similar difficulties for crystallisation. Griffin and Nelson[16] claim that they obtained a crystalline pentacetate of pinitol (pinitol has a molecular formula of $C_7H_{14}O_6$), m.p. 98°C., by this method.

Acetonation of the compound according to the method of Anderson, MacDonald and Fischer[10] by shaking the compound (0.98 g.) with dry acetone containing 1.20% hydrogen chloride (100 ml.) for five hours followed by working up the product gave crystalline diisopropylidene derivative (0.75 g.) needles (from acetone-light petroleum), m.p. 104°C., $[\alpha]_D^{31}$ (in chloroform) $= -30.5°$. (Found: C, 56.53; H, 7.805. Calcd. for $C_{13}H_{22}O_6$: C, 56.85; H, 8.05). Hydrolysis of this diisopropylidene derivative (0.23 g.) by heating on the steam bath for 30 minutes with 2N sulphuric acid (4 ml.) followed by neutralisation of the acid with barium carbonate, removal of the insoluble $BaSO_4$ by filtration, and evaporation of the neutral solution to dryness on the steam bath, gave a white crystalline residue (0.12 g.), m.p. 185°C. Recrystallised from methanol this residue had a m.p. 187°C.; its mixed m.p. with the original compound, m.p. 187°C., was undepressed. Anderson et al.[10] give a m.p. 104.5-106°C. for the diisopropylidene derivative of pinitol and $[\alpha]_D^{23} - 45.5°$ and Angyal et al.[11] record a m.p. 103-104°C. and $[\alpha]_D^{23} - 23.0°$ for this compound.

Reducing Sugar of the Leaves.—The methanolic mother liquor after separation of the sweet crystalline product from the alcohol extract of the leaves was charcoaled but there was practically no improvement of colour. The solvent was, therefore, removed whereby a syrupy brown mass (ca. 4 g.) was left. This residue was reducing as shown by a rapid reduction of Fehling's solution. It gave an osazone, m.p. 204°C., prepared by the usual method; a mixed m.p. of this osazone with an authentic sample of glucosazone, m.p. 204°C., was undepressed indicating that the reducing sugar was glucose.

Paper chromatography of this substance using ethyl acetate: water: acetic acid (3:3:1) as solvent and benzidine as spray reagent showed the presence of only glucose.

This reducing sugar has been estimated by the Schoorl's method[17] which corresponds to 0.109% on the weight of the dry leaves.

Acknowledgement.—We wish to express our gratitude to Dr. S. Siddiqui for his interest in the progress of this work and our thanks are due to Dr. M. A. Ali of these laboratories for the infrared spectra and to Mrs. Shamsun Nesa Haroon for the elemental analysis.

References

1. M. E. Ali and M. Q. Khuda, Chem. & Ind. (London), 463(1960).
2. M. Q. Khuda, M. E. Ali and M. Siddiqullah, Pakitan J. Sci. Ind. Research, **3**, 48(1960).
3. M. E. Ali and L. N. Owen, J. Chem. Soc., 2117 (1958).
4. L. J. Bellamy *The Infrared Spectra of Complex Molecules*, second edition (Methuen and Co., London, 1958).
5. W. Cocker et al., J. Chem. Soc., 2542 (1953) and the references therein.
6. P. Sengupta, S. K. Sengupta and H. N. Khastgir, Chem. & Ind. (London), 1402 (1958).
7. N. S. Narasimhan, Chem. & Ind. (London), 661 (1957).
8. R. L. Shriner, R. C. Fuson and D. Y. Curtin, *The Systematic Identification of Organic Compounds*, fourth edition, (John Wiley and Sons, Inc., New York, 1956).
9. Heilbron and Bunbury, *Dictionary of Organic Compounds* (Eyre and Spottiswoode, London), vol. 3 (1953) p. 14.
10. A. B. Anderson, D. L. MacDonald and H. O. L. Fischer, J. Am. Chem. Soc., **74**, 1479 (1952).
11. S. J. Angyal and C. G. MacDonald, J. Chem. Soc., 686 (1952).
12. H. M. Randall et al., *Infrafred Determination of Organic Structures*, (D. Van Nostrand Company, Inc., New York, 1949).
13. T. P. Hilditch, *The Chemical Constitution of Natural Fats*, third edition (Chapman and Hall Ltd., London, 1956).
14. N. Ahmad and G. Hahn, Pakistan J. Sci. Ind. Research, **2**, 55 (1959).
15. W. C. Tobie, Ind. Eng. Chem., Anal. Ed., **15**, 433 (1943).
16. E. G. Griffin and J. M. Nelson, J. Am. Chem. Soc., **37**, 1552 (1915).
17. Browne and Zerban, *Sugar Analyses*, third edition, (Chapman and Hall Ltd., London, 1955), p. 828.