CHEMICAL COMPOSITION OF *CALOTROPIS GIGANTEA*

Part IV. The Resinols of the Root Bark

By K. J. Balakrishna, P. Bhaskara Rama Murti and T. R. Seshadri

(From the Department of Chemistry, Andhra University)

Received July 16, 1945

In Part III[1] was described the study of the wax and resin components of the root bark of *Calotropis gigantea*. Besides β-amyrin, a resinol, mudarol was reported to be present in the form of ester with fatty acids of large and small chain lengths. It agreed with the general description given by the previous workers, Hill and Sirkar[2] in melting point, crystalline appearance and composition. Its properties were, however, definitely those of a resinol. It has now been shown to be a mixture consisting of two isomeric resinols, giganteol and isogiganteol. On acetylation with boiling acetic anhydride in the presence of sodium acetate, mudarol yields a product which after one crystallisation from alcohol-ether mixture melts at 195–96° agreeing with the melting point of mudarol acetate recorded by Hill and Sirkar. But, by repeated fractionation using ethyl acetate it could be separated into two fractions, (A) and (B). Fraction (A) melting at 252–53° is less soluble and is found to be identical with giganteol acetate[3] yielding giganteol on hydrolysis. Fraction (B) melts at 196–97° and yields a new alcohol melting at 177–78°. Several methods of acetylation have been examined. Milder conditions such as heating with acetic anhydride at 100° in the presence of anhydrous pyridine or treatment with acetyl chloride in the cold in the presence of anhydrous pyridine are found to be better.

That the above observations are not due to any structural change brought about by the acetylating agents has been proved in an independent way. The separation of the free alcohols, giganteol and isogiganteol from the mudarol mixture can be effected by chromatographic analysis using activated alumina and benzene-ligroin mixture. When the alcohols thus isolated are acetylated, they readily give the pure samples of giganteol and isogiganteol acetates. Mudarol seems to be an equimolecular mixture of the two alcohols.

The properties of the new alcohol, isogiganteol and of its acetate are recorded. From the composition of giganteol and isogiganteol it appears

possible that they are dihydric alcohols. But they form only monoacetates and no definite evidence for the existence of the second alcohol group has so far been obtained. The compounds do not react with 2: 4-dinitrophenylhydrazine indicating the possible absence of carbonyl groups.

Experimental

Acetylation of mudarol.

(1) *With boiling acetic anhydride.*—Mudarol (3·5 g.) was dissolved in acetic anhydride (30 c.c.), sodium acetate (5 g.) added and the mixture boiled for 3½ hours. It was then cooled, treated with ice-water, allowed to stand for 10 hours and the resulting solid was filtered. When the product was crystallised from alcohol-ether mixture, it melted at 195–96°. This substance was then repeatedly crystallised from ethyl acetate when it was obtained in two fractions. The top fraction melted at 252–53° and was identical with giganteol acetate. On hydrolysis it gave rise to giganteol melting at 223–24°. The second fraction melted at 196–97°. There was great difficulty in effecting the separation of the mixture obtained by this method of acetylation. Due to the smallness of the yields complete characterisation of the products was not attempted in this experiment.

(2) *With acetic anhydride and pyridine at* 100°.—Mudarol (2·0 g.) was dissolved in anhydrous pyridine (10 c.c.) and acetic anhydride (15 c.c.) was added. The contents were heated at 100° for 3½ hours. The mixture was then cooled and poured into a large volume of water when a colourless solid separated out. This crude product, on repeated crystallisation from ethyl acetate, could be separated into two sharp-melting crystalline products. The top fraction (I) melted at 252–53° and the fraction (II) from the mother-liquors melted at 196–97°.

Fraction I (*Giganteol acetate*).—This was a colourless solid and appeared as elongated hexagonal plates under the microscope. It dissolved in benzene, chloroform and ether but was insoluble in alcohol. When mixed with an authentic sample of giganteol acetate, the melting point was not depressed (252–53°). $[\alpha]_D^{35°}$, + 98·5° in benzene solution. (Found: C, 79·1; H, 11·1; $C_{32}H_{52}O_3$ requires C, 79·3; H, 10·7%.)

Giganteol.—The above solid (0·2 g.) was hydrolysed by boiling with 7% alcoholic potash. When the reaction mixture was concentrated and allowed to cool slowly, crystals of the free alcohol separated out. After one crystallisation from alcohol it melted at 223–24° and appeared as rectangular rods under the microscope. There was no change either in melting point or crystal structure on further crystallisation. It did not form a hydrazone

with 2-4-dinitrophenyl-hydrazine. $[\alpha]_D^{35°}$, + 111·2° in benzene. The colour reactions were the same as those of giganteol. The mixed melting point with an authentic sample of giganteol was undepressed.

Fraction II (*Isogiganteol acetate*).—This was a colourless solid and appeared as tiny plates under the microscope. It was soluble in ether, chloroform and benzene in the cold and in boiling alcohol. It gave a pink colour with the Liebermann-Burchard reagent and a yellow solution exhibiting green fluorescence with the Salkowski reagent. (Found: C, 79·8; H, 10·8; $C_{32}H_{52}O_3$ requires C, 79·3; H, 10·7%.) The mixed melting point of this compound with giganteol acetate was found to be depressed (175–80°). $[\alpha]_D^{35°}$, + 96·2° in benzene solution.

Isogiganteol.—The above compound (0·2 g.) was hydrolysed by boiling with 7% alcoholic potash. When the reaction mixture was concentrated, a colourless solid separated out. It was crystallised from alcohol, when a glistening crystalline solid (hexagonal plates) melting at 177–78° was obtained. With the Liebermann-Burchard and Salkowski reagents it gave the same colour reactions as giganteol and the calotropeols. There was no change either in melting point or crystal structure on further crystallisation. It did not react with 2-4-dinitrophenyl-hydrazine. $[\alpha]_D^{35°}$, + 97·4° in benzene solution. (Found: C, 82·0; H, 11·1; $C_{30}H_{50}O_2$ requires C, 81·4; H, 11·3%.)

(3) *With acetyl chloride and pyridine at* 0°.— Mudarol (0·5 g.) was dissolved in anhydrous pyridine (10 c.c.) and the solution was cooled to 0°. Acetyl chloride (2 c.c.) was added drop by drop, all the while taking care to see that the contents were stirred well and that the temperature was maintained at 0°. After the addition of all the acetyl chloride the mixture was heated on a boiling water-bath for 2 minutes and poured into a large volume of water. The crude reaction product which separated out, was repeatedly crystallised from ethyl acetate when two fractions, one melting at 252–53° and the other melting at 196–97° were obtained just as in the previous acetylation and the products were the same. The yields in methods 2 and 3 were equally good and the separation was not very difficult. The two resinols appeared to be present approximately in equal amounts.

Chromatographic analysis of mudarol.

Mudarol (3·5 g.) was dissolved in 150 c.c. of benzene-ligroin (b.p. 60–80°) mixture (5 : 3) and the solution allowed to pass down a column (20 cm. by 2½ cm.) of activated alumina (B.D.H.) under gentle suction. The column was then successively washed with the same solvent using 100 c.c. each time.

Each fraction was collected separately and the dissolved solid, by crystallisation from the same solvent, obtained in two crops, the final mother liquor being discarded. Details of the analysis are given below:

Serial No.	Fraction	Yield in grams	I Crop		II Crop
			Melting point	Crystal structure	Melting point
1	Original filtrate	Nil	..	..	..
2	Benzene-ligroin (5 : 3) ..	Nil	..	..	..
3	do. ..	Nil	..	..	..
4	do. ..	0·2	176–77°	Long and soft needles	176–77°
5	Benzene-ligroin (6 : 2) ..	0·65	176–77°	do.	177–78°
6	do. ..	0·52	177–78°	Hexagonal plates	176–77°
7	do. ..	0·68	177–78°	Elongated plates and flat needles	179–80°
8	Benzene-ligroin (6 : 1) ..	0·41	185–86°	do.	196–98°
9	do. ..	0·30	189–90°	Broad plates and needles	200–01°
10	do. ..	0·33	193–94°	do.	200–01°
11	Benzene	0·21	197–98°	do.	209–10°
12	do.	0·06	203–04°	do.	209–10°
		3·36			

Crop I of fractions 4, 5 and 7 and crop II of fractions 4, 5, 6 and 7 (*i.e.*, fractions melting below 180°) were put together as 'A'. Crop I of fraction 6 had a remarkably uniform crystal structure and seemed to be quite pure. Hence it was kept separately. Its mixed melting point with isogiganteol was undepressed. On acetylation using acetic anhydride and pyridine it gave a pure sample of isogiganteol acetate melting at 196–97° after one crystallisation from ethyl acetate. Crop I of fractions 8, 9 and 10 (*i.e.*, those melting between 185–93°) was put together and marked 'B'. Crop I of fractions 11 and 12 and crop II of fractions 8, 9, 10, 11 and 12 (*i.e.*, those melting above 197°) were put together as 'C'.

Analysis of 'A'.—The substance was dissolved in 50 c.c. of benzene-ligroin mixture (5 : 3) and was passed down a column of activated alumina (10 cm. by ½ cm.). Fractional elution was done using 25 c.c. of the same solvent in each case.

Serial No.	Fraction	Melting point	Crystal structure	Yield in grams
1	Original filtrate	..	..	Nil
2	Benzene-ligroin (5 : 3)	180–82°	Needles	0·10
3	do.	170–73°	Plates and needles	0·15
4	do'	177–78°	Hexagonal plates	0·10
5	do.	177–78°	do.	0·10

The last two fractions were mixed together and the substance was found to be identical with crop I of fraction 6 and with isogiganteol in melting point and crystal structure. On acetylation with acetic anhydride and pyridine and after one crystallisation of the reaction product from ethyl acetate, a pure sample of isogiganteol acetate melting at 196–97° was obtained.

Substance 'B'.—On acetylation using the same method as above, a crystalline solid melting at 252–53° was obtained after one crystallisation from ethyl acetate and it was identical with giganteol acetate in melting point and crystal structure. This fraction seemed to contain giganteol as the major component.

Analysis of 'C'.—The substance was dissolved in 50 c.c. of benzene-ligroin (5: 3) mixture and passed down a column of activated alumina (10 cm. by ½ cm.). Fractional elution was done using 25 c.c. of the solvent each time.

Serial No.	Fraction	Melting point	Yield in grams
1	Original filtrate	..	Nil
2	Benzene-ligroin (5 : 3)	193–95°	0·05
3	do.	185–87°	0·10
4	do.	178–81°	0·10
5	do.	195–97°	0·08
6	do.	220° (sintering at 216°)	0·10

The last fraction when crystallised from benzene melted sharp at 223–24° and appeared as rectangular rods under the microscope. A mixed melting point of this substance with giganteol was found to be undepressed. Acetylation of the substance using acetic anhydride and pryidine gave a product which on crystallisation from ethyl acetate melted at 252–53°. It was identical with giganteol acetate in melting point and crystal structure.

Summary

Mudarol, the chief resinol component of the root bark of *Calotropis gigantea*, has been separated into two isomeric compounds by fractional crystallisation of the acetates and also by chromatographic adsorption analysis of the free alcohols. Giganteol which was first obtained from the stem bark is one of them and a new triterpene alcohol, isogiganteol is the other. The properties and reactions of the new resinol are described. Though giganteol and isogiganteol ($C_{30}H_{50}O_2$) have two oxygen atoms they form only monoacetates. They do not combine with dinitrophenyl-hydrazine.

References

1. Murti and Seshadri .. *Proc. Ind. Acad. Sci.*, A, 1945, **21**, 147.
2. Hill and Sirkar .. *J. C. S.*, 1915, 1437.
3. Murti and Seshadri .. *Proc. Ind. Acad. Sci.*, A, 1945, **21**, 17.