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STUDIES ON THE BARKS OF THE FAMILY SALICACEAE—XVI[1]

THE STRUCTURE OF SALIREPOSIDE

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Abstract—Complete methylation of salireposide followed by hydrolysis to yield 2,3,4,6-tetra-*O*-methylglucose as the only sugar suggested that the long-accepted structure for salireposide as hydroxypopulin (I) was incorrect. The mass spectral fragmentation pattern proved that the benzoyl substitution of salireposide was not on the glucose moiety and that salireposide must be 2-benzoyloxymethyl-4-hydroxyphenyl-β-D-glucoside (II). This was confirmed by its enzymic hydrolysis with β-glucosidase to glucose and benzoylgentisyl alcohol (VI).

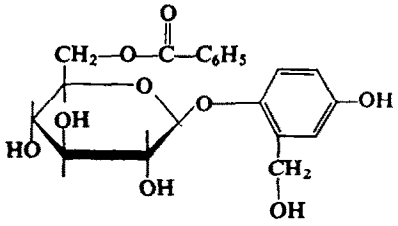
IN ONE of our early studies on the barks of the family Salicaceae,[2] we isolated salireposide from the bark of *Populus tremuloides*. At that time the structure of salireposide had been demonstrated to be a monobenzoate of 4-hydroxy-2-hydroxymethylphenyl-β-glucoside and assumed to be hydroxypopulin (I). Because the location of benzoyl substitution had not been proven, attempts were made to locate the position of the benzoyl group by the methylation procedure employed so successfully for determining the structure of tremuloidin[3] and grandidentatin.[4] Attempted methylations of salireposide by the traditional Purdie method gave only dark-colored tars, and the products were useless for structure determination. However, salireposide was completely methylated after only one treatment by the Kuhn, Trischmann and Löw[5] dimethylformamide modification of the Purdie procedure. Hydrolysis of the product with methanolic hydrogen chloride yielded only 2,3,4,6-tetra-*O*-methylglucose, suggesting that the benzoyl substitution was not on the glucose moiety. Nevertheless, because of the possibility of hydrolysis of the benzoyl during the methylation with subsequent methylation of the liberated hydroxyl group, and because of the general acceptance by previous investigators of the glucose-*O*-benzoyl structure, we hesitated to accept this experiment as proof that benzoyl substitution was not on the glucose unit.

Meanwhile, Thieme[6] reported the methylation of salireposide by the Kuhn, Trischmann and Löw procedure and hydrolysis of the methylated product to yield 2,3,4-tri-*O*-methylglucose as the chief product, as indicated by paper chromatography. On the basis of this

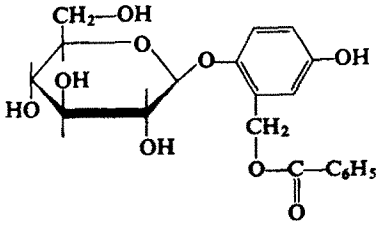
[1] The conclusions of this paper were reported in a preliminary publication in *Tetrahedron Letters* **20**, 1869 (1967).
[2] I. A. PEARL and F. S. DARLING, *J. Org. Chem.* **24**, 1616 (1959).
[3] I. A. PEARL and S. F. DARLING, *J. Org. Chem.* **24**, 731 (1959).
[4] I. A. PEARL and S. F. DARLING, *J. Org. Chem.* **27**, 1806 (1962).
[5] R. KUHN, H. TRISCHMANN and I. LÖW, *Angew. Chem.* **67**, 32 (1955).
[6] H. THIEME, *Naturwissenschaften* **51**, 291 (1964).

evidence, Thieme concluded that the benzoyl group must have occupied the 6-*O*-glucose position and, therefore, salireposide had structure I.

The discrepancy between our methylation results and those of Thieme led us to investigate the structure of salireposide by an unambiguous method of degradation and by means of mass spectrometry. While this work was in progress, Thieme[7] reported that prolonged methylation of salireposide gave, on subsequent hydrolysis with acid, 2,3,4,6-tetra-*O*-methylglucose instead of the 2,3,4-tri-*O*-methylglucose reported earlier.[6] In addition, Thieme hydrolysed salireposide with emulsin to yield glucose and an oil which was hydrolysed further by acid or alkali to yield benzoic acid. He noted that gentisyl alcohol could be identified by lipase hydrolysis of the aglucone. On the basis of this evidence, Thieme proposed structure II for salireposide.



(I)



(II)

Other work in our laboratory on the mass spectrometry of *Populus* and *Salix* glucosides[8] indicated that the primary fragmentation of the acetates of these glucosides always take place at the C-1 bond so that it is possible to determine whether or not the glucose moiety of the glucoside is substituted. Accordingly, the mass spectrum of salireposide pentaacetate (III) was determined and found to contain the following major and important *m/e* peaks, with relative *m/e* intensity for each peak noted in parentheses: 616 (<0·1), 331 (26), 169 (76), 109 (49), 105 (22), 77 (8), and 43 (100).

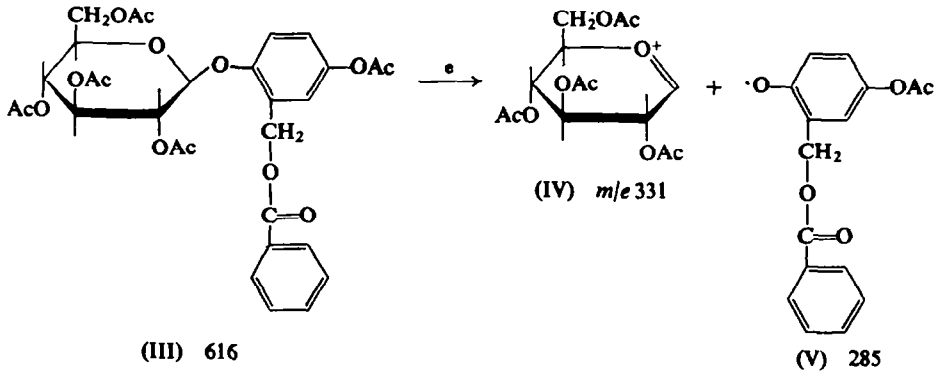
The weak molecular ion peak for III (*m/e* 616) is characteristic of all glucoside acetate spectra investigated earlier.[8] The first prominent peak at *m/e* 331 corresponds to the tetra-acetylglucose oxonium ion (IV), the primary fragmentation ion obtained by Biemann and co-workers[9] in the mass spectra of glucose pentaacetate and the acetates of methyl and phenyl glucosides and by us in earlier studies on the acetates of glucosides containing no acyl substitution on the glucose moiety.[8] The *m/e* 331 peak also corresponds to the molecular ion less the benzoylgentisyl alcohol radical (V). Thus, the primary fragmentation pattern for III is that of Scheme 1.

The important peaks at *m/e* 169 and 109 are due to secondary fragmentation of the primary ion by loss of acetic acid or ketene and that at *m/e* 43 is due to the acetyl ion, as noted in earlier studies.[8,9] The peaks at *m/e* 105 and 77 are due to the benzoyl and phenyl ions, respectively, from fragmentation of the aglucone.

If salireposide had structure I, primary fragmentation of its pentaacetate should have given a prominent peak at *m/e* 393 corresponding with the monobenzoyltriacetylglucose oxonium ion found in the mass spectra of acetates of all glucosides containing benzoyl substitution in the glucose unit.[8] No trace of a peak was obtained in the spectrum of III at

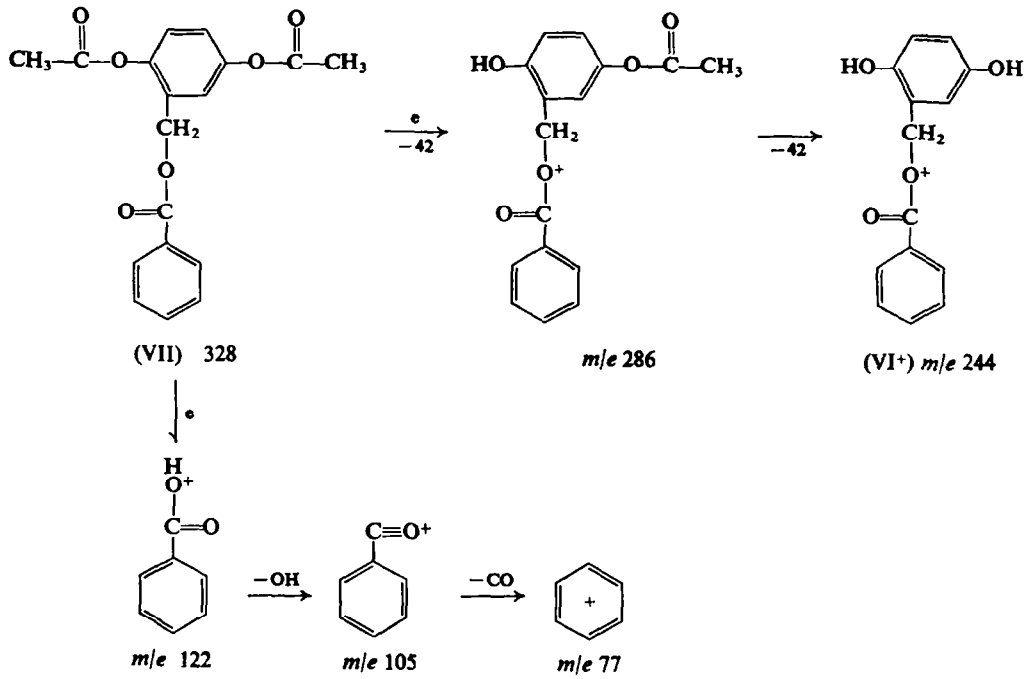
[7] H. THIEME, *Pharmazie* **21**, 769 (1966).
[8] I. A. PEARL and S. F. DARLING, *Phytochem*, in press.
[9] K. BIEMANN, D. C. DEJONGH and H. K. SCHNOES, *J. Am. Chem. Soc.* **85**, 1763 (1963).

m/e 393, and it became apparent that salireposide could not be I. The fact that salireposide contains a free phenolic hydroxyl group indicates that salireposide must have structure II.



(III) 616 (IV) *m/e* 331 (V) 285

SCHEME 1.



(VII) 328 *m/e* 286 (VI⁺) *m/e* 244

m/e 122 *m/e* 105 *m/e* 77

SCHEME 2.

Structure II was confirmed by its enzymic degradation with β-glucosidase to glucose and benzoylgentisyl alcohol (VI). The latter was characterized by analysis and mass spectrometry of the crystalline diacetate (VII), the mass spectrum of which contained the following major and important *m/e* peaks, with relative *m/e* intensity for each peak noted in parentheses: 328 (1·5), 286 (19), metastable 249·5, 244 (26), metastable 208, 122 (100), 105 (82), 77 (37), and 43 (98). The molecular ion peak at *m/e* 328 for VII is the calculated molecular weight. The spectrum contains two prominent peaks at *m/e* 286 and *m/e* 244 which are due to the

sequential loss of the two phenolic acetyl groups as ketene from the molecular ion. This conclusion is confirmed by the appearance in the spectrum of two metastable peaks which indicate that the parent of the *m/e* 286 (328–42) is the molecular ion at *m/e* 328 and that the peak at *m/e* 244 (286–42) has the peak at *m/e* 286 as its parent. Other peaks in the spectrum are due to the following ions: *m/e* 122 (benzoic acid), *m/e* 105 (benzoyl), *m/e* 77 (phenyl), and *m/e* 43 (acetyl). The fragmentation pattern for VII is pictured in Scheme 2.

EXPERIMENTAL

Methylation of Salireposide

1 g of chromatographically pure salireposide in 25 ml of dimethylformamide in a pressure bottle was treated with 6 ml MeI and 6 g silver oxide, and the mixture was shaken at 25° for 16 hr.[5] The reaction mixture was centrifuged, and the residue was washed with a little dimethylformamide by centrifugation. The pale-yellow solution was diluted with water, and the precipitated silver salts were dissolved with excess KCN solution. The aqueous mixture was extracted with $CHCl_3$ (4 × 50 ml), the $CHCl_3$ solution washed with water (4 × 75 ml), dried and evaporated under reduced pressure to leave a pale syrup which failed to crystallize. The syrupy methylated salireposide was hydrolysed with methanolic hydrogen chloride, and the hydrolysate was examined by paper chromatography as described earlier[3] and contained only 2,3,4,6-tetra-*O*-methyl-glucose.

Salireposide Pentaacetate (III)

Salireposide was acetylated with acetic anhydride and pyridine, and the product was recrystallized from ethanol to give III, melting at 125–126° not depressed by admixture with authentic III.[2] Its i.r. spectrum contained bands at 2·92, 3·41, 5·71, 5·78, 6·22, 6·66, 6·87, 6·98, 7·29, 7·60, 7·85, 8·12, 8·40, 8·64, 8·91, 9·06, 9·23, 9·37, 9·60, 10·16, 11·02, 12·14, 12·40, and 14·14 μ.

Enzymic Hydrolysis of Salireposide

A suspension of 1 g of salireposide in 150 ml 0·01 M NaOAc brought to pH 5·0 with acetic acid was heated almost to boiling to form a clear solution and allowed to cool to 40°. The clear solution was treated with stirring with 150 mg of β-glucosidase at 40° for 1 hr. During this time, the solution became progressively cloudy, and a curdy precipitate separated. The mixture was extracted twice with ether, and the ether was washed twice with dil $NaHCO_3$ and dried. The ether was removed to leave 0·43 g (75 per cent) of benzoylgentisyl alcohol (VI) as a colorless syrup which crystallized upon standing. It was recrystallized from petrol. ether (b.r. 30–60°) to give colorless crystals melting at 102–103° and having the following bands in its i.r. spectrum: 3·00, 5·90, 6·23, 6·30, 6·64, 6·89, 7·26, 7·60, 7·81, 8·37, 8·50, 8·67, 8·96, 9·13, 9·35, 9·75, 9·99, 10·46, 10·87, 11·52, 12·27, 12·85, 13·26, 14·09, and 14·64 μ.

Benzoylgentisyl Alcohol Diacetate (VII)

Benzoylgentisyl alcohol (VI) was acetylated with pyridine and acetic anhydride, and the product was recrystallized from methanol containing a little water to yield colorless needles melting at 62·5–63° and having the following bands in its i.r. spectrum: 5·69, 5·82, 6·23, 6·68, 6·89, 6·99, 7·30, 7·61, 7·86, 8·27, 8·47, 8·72, 8·98, 9·14, 9·35, 9·62, 9·83, 10·36, 10·50, 10·65, 10·83, 11·58, 11·89, 12·91, 14·13, and 14·64 μ. (Found: C, 65·75; 66·00; H, 4·83, 4·87. Calc. for $C_{18}H_{16}O_6$: C, 65·85; H, 4·91%.)

Saponification of Benzoylgentisyl Alcohol

A solution of 0·4 g of (VI) in a few milliliters of ethanol under N_2 was treated with 3 ml N NaOH, and the resulting black solution was boiled under reflux for a few minutes. The mixture was cooled, saturated with CO_2 to pH 7·0, and extracted with ether. The aqueous raffinate was acidified further with HCl and extracted with ether. This ether was evaporated, and the resulting solid was recrystallized from petrol. ether (b.r. 30–60°) to give colourless needles, melting at 120–121° not depressed on admixture with authentic benzoic acid. The aqueous raffinate left after ether extraction was freed of cations by passing through a column of Amberlite IR-120 resin. The effluent was concentrated and the syrupy residue was treated with methanol and *p*-nitroaniline[4] to yield yellow needles of *p*-nitroaniline-*N*-β-D-glucoside, melting at 183–185° not depressed by admixture with authentic material.[10]

Acknowledgements—All melting points are uncorrected. Analyses were performed by Micro-Tech Laboratories, Skokie, Illinois. I.r. spectra were determined in KBr discs by Mr. Lowell Sell of The Institute of Paper Chemistry Analytical Department with a Perkin-Elmer model 21 recording spectrophotometer. Mass spectra were determined by Morgan-Schaffer Corp., Montreal, Quebec, Canada, on a double focusing Hitachi instrument.

[10] F. WEYGAND, W. PARKOW and P. KUHNER, *Chem. Ber.* **84**, 594 (1951).