57. *Constituents of the Leaves of Certain* Leucadendron *Species. Part I. Leucodrin.*

By WILLIAM SAGE RAPSON.

Leucodrin has been isolated from the leaves of *Leucadendron concinnum*, *L. adscendens*, and *L. Stokoei*, and a preliminary study of its constitution made. It is a dilactone, $C_{15}H_{16}O_8$, containing one phenolic and three alcoholic hydroxyl groups. The phenolic hydroxyl is present in a *p*-hydroxyphenylethyl group. *Dibromoleucodrin* and *dichloroleucodrin* show unexpected properties. Both are dilactones containing one acidic group (titration experiments) and three reactive hydrogen atoms (Zerewitinow test). They would be expected, therefore, to give diacyl derivatives. Actually, tetra-acyl derivatives $C_{15}H_{10}O_8X_2Ac_4$ (X = Cl or Br) are obtained, in which the acidic properties of the original compounds have disappeared.

Rutin has been identified as a further constituent of the above *Leucadendron* species, having been isolated in large amounts from *L. Stokoei*.

LEUCODRIN was first isolated from the leaves of *L. concinnum* by Meiring-Beck (*Pharm. J.*, 1886, **17**, 327, 408), and later by Schuchardt (*ibid.*, p. 755), by Merck (*Merck's Ber.*, 1895, 3), and by Hesse (*Annalen*, 1896, **290**, 314). Besides leucodrin Merck isolated a glycoside leucoglycodrin from *L. concinnum*, but no other constituents of the leaves were reported. Both these substances are very bitter, and their presence in the leaf is probably responsible for the use of extracts of the leaves as a quinine substitute in the past (cf. Watt and Breyer-Brandwijk, "The Medicinal and Poisonous Plants of Southern Africa," Edinburgh, 1932). For the present work, leucodrin was obtained from the leaves of three species of *Leucadendron*—from *L. concinnum*, *L. adscendens*, and *L. Stokoei*.

Little information with regard to its chemical structure came from the experiments of Hesse and Merck on leucodrin (*loc. cit.*). Merck favoured a formulation $C_{15}H_{16}O_8$,

with eight hydroxyl groups (formation of an octa-acetyl derivative), and Hesse from later experiments stated that leucodrin had the formula $C_{18}H_{20}O_9$ and gave a triacetyl derivative. The analyses and molecular-weight determinations presented here indicate the formula $C_{15}H_{16}O_8$. Titration experiments establish the presence of two lactone groups in the molecule; this is confirmed by the preparation of the corresponding *dihydroxydiamide*, *dihydroxybisethylamide*, and *dihydroxybisbenzylamide*. Of the remaining four oxygen atoms, Zerewitinow estimations indicate that three are in hydroxyl groups. Evidence for the presence of a fourth hydroxyl group, however, comes from two directions. First, acetylation of leucodrin yields a crystalline acetyl leucodrin, and the repeated acetyl determinations on this compound (C and H values are not significant) indicate that it is a *tetra-acetyl leucodrin*. Propionylation, butyrylation, and benzoylation experiments under various conditions all fail to yield crystalline products; mixtures of products may result. Secondly, leucodrin condenses with acetone to yield an iso*propylidene leucodrin* which gives a *diacetyl* iso*propylidene leucodrin* on acetylation, and yields two molecules of methane with the Grignard reagent; thus indicating the original presence of four hydroxyl groups.

These results are confirmed by the study of *leucodrin methyl ether* and *bromoleucodrin methyl ether*. The methyl ether is formed from leucodrin by carefully controlled treatment with diazomethane and is oxidised by potassium permanganate to give anisic acid. It contains two lactone groups, and the Zerewitinow test reveals the presence of two hydroxyl groups. An iso*propylidene-leucodrin methyl ether*, however, still gives a *mono-acetyl* derivative and yields one molecule of methane with the Grignard reagent. Bromoleucodrin methyl ether is formed by the direct action of bromine on leucodrin methyl ether. It is oxidised by potassium permanganate to 3-bromoanisic acid and contains two lactone groups and two reactive hydrogen atoms. Its iso*propylidene* derivative, however, also yields one molecule of methane with the Grignard reagent. Both of these compounds resemble leucodrin itself in failing to give crystalline acyl derivatives—in these cases even the product of acetylation fails to crystallise.

Leucodrin is extremely stable to the action of both acids and alkalis. Boiling concentrated hydrochloric acid has no action on it, and even after vigorous treatment with 15% sodium hydroxide solution it can be regenerated unchanged on acidification of the mixture. Hesse (*loc. cit.*) reported as one of the properties of leucodrin that it gave a yellow coloration with alkali. Completely purified leucodrin, however, gives no colour with alkali, the colour reaction of the crude material being due to traces of the flavonol glycoside rutin, which can be obtained in quantity from the leaves of *L. Stokoei*.

Potash fusion of leucodrin yields a mixture of *p*-hydroxybenzoic acid, acetic acid, and β-*p*-hydroxyphenylpropionic acid. The first two result from the last on potash fusion, so the presence of the skeleton (*p*) $OH{\cdot}C_6H_4{\cdot}CH_2{\cdot}CH_2{\cdot}C$ in the leucodrin molecule is all that can be deduced. Alkaline solutions of leucodrin couple with diazonium salts, and with bromine leucodrin yields a *dibromoleucodrin*, $C_{15}H_{14}O_8Br_2$. The latter reaction proceeds quantitatively, and titration of leucodrin with bromide–bromate mixture provides the most accurate method of determining its molecular weight. The product of the methylation of dibromoleucodrin with diazomethane is oxidised to 3 : 5-dibromoanisic acid by potassium permanganate. Dibromoleucodrin, however, possesses unexpected properties. From titration experiments it contains one acidic and two lactone groups. The acidic group cannot be esterified catalytically. Zerewitinow estimations indicate the presence of three reactive hydrogen atoms. Since one of these is in the acidic group, we should expect to obtain diacyl derivatives on acylation. But dibromoleucodrin yields *tetra-acetyl* and *tetrapropionyl dibromoleucodrins*, $C_{15}H_{10}O_8Br_2(COR)_4$ ($R = CH_3$ and $CH_2{\cdot}CH_3$), which are no longer acidic; the acidic group disappears during acylation. Both compounds, however, are hydrolysed to dibromoleucodrin by alkalis. This series of changes is confirmed by the study of *dichloroleucodrin* and *tetra-acetyl dichloroleucodrin*, which possess corresponding properties.

Attempts to obtain degradation products by the action of nitric acid have not been successful; the only crystalline product isolated gives analytical figures corresponding to a *dinitroleucodrin*, $C_{15}H_{14}O_8(NO_2)_2$. It is soluble in sodium bicarbonate solution. Further degradation experiments are in progress.

Experimental.

Extraction of Leucodrin.—The leaves were dried, ground, and extracted continuously with hot alcohol till free from bitter material. The alcohol was then evaporated from the extract, the residue dissolved in water, and the solution clarified by the addition of lead subacetate solution. On evaporation of the clear filtrate from the lead precipitation, the leucodrin crystallised. In some cases a yellow flocculent precipitate separated from the clear solution if this was kept for 24 hours before evaporation. In such cases a fairly clear separation of the yellow material from the leucodrin could be obtained by cooling the dilute aqueous solution, and filtering off the pigment before evaporation in a vacuum. The final viscous mother-liquor after the crystallisation of the leucodrin contained sugars which yielded phenylglucosazone on treatment with phenylhydrazine in the usual way. By this procedure, *L. concinnum* (female plant) yielded 16% as leucodrin, besides traces of pigment; *L. adscendens* (female plant) yielded 20% as leucodrin, and appreciable amounts of pigment; and *L. Stokoei* (female plant) yielded 16% as leucodrin and 10% as pigment (all the yields are calculated on the dry weight of the leaf). Slight variations in the yields depended on the sex of the plant and the season of the year at which the specimen was collected. In no case was any attempt made to isolate the glycoside leucoglycodrin.

Identification of the Yellow Pigment as Rutin.—As obtained by the above procedure, the yellow material consisted of a mass of interlaced crystals which was very retentive of water on the filter. The mass was dried, powdered, and dissolved in a large quantity of ethyl alcohol, the excess of which was evaporated once solution had been achieved. From the concentrated solution, after treatment with decolorising charcoal, the pigment separated in a crystalline form easy to filter. When pure, the crystals coalesced to opaque droplets in the melting point tube at 184°, and these did not clear till 193°. Even on prolonged heating no meniscus formed (Found: C, 48·5, 48·4; H, 5·6, 5·4; loss in weight at 160°, 8·5. Calc. for $C_{27}H_{30}O_{16},3H_2O$: C, 48·7; H, 5·4; H_2O, 8·1%). That it was a glycoside of quercitin was indicated by the production of phloroglucinol and protocatechuic acid on potash fusion, and this was confirmed by its quantitative hydrolysis (cf. Smith, J., 1898, **73**, 699) to yield quercetin (Found: quercetin, 45·0. Calc. for $C_{27}H_{36}O_{19}$: quercetin, 45·5%). Its probable identity with rutin was established by the isolation (cf. Perkin, J., 1910, **97**, 1776) of the phenylosazones of glucose and rhamnose from the hydrolysis mixture. As the osazone of rhamnose was difficult to obtain pure, the rhamnose itself was isolated by the method adopted by Perkin (*loc. cit.*). To make certain of this identity, a sample of the substance was treated with excess of diazomethane (Attree and Perkin, J., 1927, 238) and from the hydrolysis of the product a substance with the same characteristics as 5 : 7 : 3′ : 4′-tetramethyl quercetin was isolated.

Leucodrin.—Leucodrin, recrystallised from water, formed elongated prisms of rectangular outline, m. p. 212—212·5°. It gave no coloration with ferric chloride and when completely freed from pigment it gave no yellow colour with acid or alkali. It was sparingly soluble in non-hydroxylic solvents except dioxan and ethyl acetate, and crystallised unchanged from boiling concentrated hydrochloric acid [Found: C, 55·6, 55·6; H, 4·9, 5·2; *M* (Rast), 376; OMe, nil; reactive H (Zerewitinow estimation in pyridine), 0·87, 0·92. Calc. for $C_{15}H_{16}O_8$ (three reactive hydrogens): C, 55·5; H, 5·2%; *M*, 324; reactive H, 0·93%]. Leucodrin has specific rotatory power $[\alpha]_D$ − 15·45° (from Merck, *loc. cit.*). In aqueous solution, it neutralised baryta slowly in the cold. Titrations were carried out by the addition of excess of N/20-baryta and back-titration in the hot; the end-point was rather indefinite [Found: equiv., 155. Calc. for $C_{15}H_{16}O_8$ (two lactone groups): equiv., 162].

iso*Propylidene Leucodrin.*—Leucodrin (2 g.) was dissolved in dry acetone (150 c.c.), and dry hydrogen chloride passed in until its concentration was 0·5%. The mixture was left at room temperature overnight, the mineral acid then neutralised with lead carbonate, and the filtered solution evaporated; the residue crystallised on scratching. It was dissolved in alcohol, and water added in suitable amount; on freezing, iso*propylidene leucodrin* separated in colourless prisms, m. p. 229·5—231·5° [Found: C, 58·9; H, 5·5; reactive H (Zerewitinow estimation in pyridine), 0·60. $C_{18}H_{20}O_8$ (two reactive hydrogens) requires C, 59·3; H, 5·5; reactive H, 0·55%].

Diacetyl iso*propylidene leucodrin* separated as an oil when water was added to a solution of *iso*propylidene leucodrin (2 g.) in pyridine (3 g.) and acetic anhydride (1·5 g.) which had been kept overnight; it crystallised in contact with ethyl alcohol, and separated from this solvent in elongated prisms, m. p. 168—169° (Found: C, 58·6; H, 5·3. $C_{22}H_{24}O_{10}$ requires C, 58·9; H, 5·4%).

The Action of Ammonia, Ethylamine, and Benzylamine on Leucodrin.—Leucodrin (1 g.) was added to a dry saturated solution of ammonia in methyl alcohol (10 c.c.); after 24 hours the corresponding *dihydroxydiamide* crystallised. Recrystallised from methyl alcohol, it formed long matted needles, m. p. 175—176° with evolution of gas. Freed from solvent, the material was very hygroscopic (Found: C, 50·5; H, 6·5; N, 7·8. $C_{15}H_{22}O_8N_2$ requires C, 50·3; H, 6·2; N, 7·8%).

In a similar manner ethylamine and leucodrin yielded the corresponding *dihydroxybisethylamide*, which crystallised from water in prisms, m. p. 184—185° with evolution of gas (Found: C, 55·5; H, 7·7; N, 7·0. $C_{19}H_{30}O_8N_2$ requires C, 55·2; H, 7·3; N, 6·8%).

The *dihydroxybisbenzylamide* was recrystallised from aqueous alcohol. It effervesced and melted when placed in a bath above 130°, solidified, and remelted at 212°. When, however, the substance was placed in a bath below 130°, no visible effervescence occurred, the substance remaining unmolten till 212° (Found for substance dried in a vacuum at 80°: C, 65·0; H, 6·5; N, 4·9. $C_{29}H_{34}O_8N_2$ requires C, 64·7; H, 6·4; N, 5·2%).

Acetylation.—Leucodrin was heated with acetic anhydride alone for 15 hours, or in the presence of anhydrous sodium acetate or pyridine. The product, which could be hydrolysed back to leucodrin, crystallised from acetic acid in prisms, m. p. 191—192° [Found: C, 56·0, 55·9; H, 5·1, 5·0; CH_3·CO, 36·0. $C_{15}H_{12}O_8(CO{\cdot}CH_3)_4$ requires C, 56·1; H, 4·9; CH_3·CO, 35·0%]. It appears to be a *tetra-acetyl leucodrin.*

Leucodrin Methyl Ether.—Leucodrin (10 g.) was dissolved in dry methyl alcohol (60 c.c.) and treated with an ethereal solution of diazomethane (from nitrosomethylurea, 15 g.). After 12 hours any excess of diazomethane was destroyed and the solvents were evaporated. The viscous residue crystallised in only a few experiments. In difficult cases, the product could sometimes be obtained crystalline by boiling the oily product in alkaline solution for a few minutes, acidifying the solution, and evaporating it. Radiating clusters of crystals then separated; these melted indefinitely above 90°, the melt solidifying and remelting at 174—175°. This behaviour was due to water of crystallisation, which was gradually removed above 100° [Found: C, 54·3, 54·0; H, 5·7, 6·0; H_2O (lost at 100°), 5·1; reactive H, 1·01; OMe, 8·6. $C_{16}H_{18}O_8,H_2O$ (four reactive hydrogens) requires C, 53·9; H, 5·6; H_2O, 5·4; reactive H, 1·12; OMe, 8·7%]. On oxidation with potassium permanganate solution, this *methyl* ether yielded anisic acid, thus proving that the methyl group had been introduced on a phenolic hydroxyl group. It was insoluble in sodium bicarbonate solution, but soluble in caustic alkali [Found: equiv. by back-titration in the hot, 170. $C_{15}H_{18}O_8$ (two lactone groups) requires equiv., 169]. Attempts to prepare an acetyl derivative in the usual way led to oily viscous products which could not be purified. On potash fusion, it yielded, not anisic acid as expected, but a mixture of *p*-hydroxybenzoic acid and β-*p*-hydroxyphenylpropionic acid.

iso*Propylidene-leucodrin Methyl Ether.*—Leucodrin methyl ether was treated with acetone and hydrogen chloride as in the preparation described for *iso*propylidene leucodrin. The viscous product was taken up in acetone, and water added; on cooling in ice, iso*propylidene-leucodrin methyl ether* crystallised slowly in prisms, m. p. 161·5—162·5° [Found: C, 60·9; H, 5·7; reactive H, 0·3. $C_{19}H_{22}O_8$ (one reactive H) requires C, 60·4; H, 5·8; reactive H, 0·26%]. In later experiments, this substance was obtained in better yields by the action of diazomethane on *iso*propylidene leucodrin.

Acetyl-iso*propylidene-leucodrin Methyl Ether.*—*iso*Propylidene-leucodrin methyl ether (1 g.) was treated with a mixture of pyridine (1·5 c.c.) and acetic anhydride (0·7 c.c.) in the cold. After 12 hours water was added, and the *product* recrystallised from ethyl alcohol. It separated in prisms of hexagonal outline, m. p. 160·5—161·5° (Found: C, 60·0; H, 6·0. $C_{21}H_{24}O_9$ requires C, 60·0; H, 5·7%).

Bromoleucodrin methyl ether, obtained from leucodrin methyl ether by the action of bromine in methyl-alcoholic medium, crystallised from aqueous alcohol in colourless prisms of rectangular outline, m. p. 243—244° after sintering at 238°. It was soluble in caustic alkali but insoluble in sodium bicarbonate solution [Found: C, 46·3; H, 4·1; Br, 18·3; equiv., by back-titration in the hot, 205. $C_{16}H_{17}O_8Br$ (two lactone groups) requires C, 46·0; H, 4·1; Br, 19·1%; equiv., 208·5].

iso*Propylidene-bromoleucodrin methyl ether*, recrystallised from methyl alcohol, formed white plates, m. p. 108—110° (Found: C, 49·6; H, 4·6; Br, 17·3. $C_{19}H_{21}O_8Br$ requires C, 49·9; H, 4·6; Br, 17·5%).

Fusion of Leucodrin with Alkalis.—Leucodrin (3 g.) was mixed with sodium hydroxide (9 g.) and potassium hydroxide (6 g.), water added, and the mixture stirred at 220° for 25 minutes. The cooled melt was dissolved in as little water as possible, the solution acidified

with sulphuric acid and extracted with ether, the extracts dried, and the ether evaporated. The solid residue smelt strongly of acetic acid and of phenol. By a long series of fractional crystallisations from water it was separated into *p*-hydroxybenzoic acid and β-*p*-hydroxyphenylpropionic acid, m. p. 128·5—129·5° (Found: C, 65·1; H, 6·1. Calc. for $C_9H_{10}O_3$: C, 65·1; H, 6·0%). The latter was identified by a mixed m. p. test with an authentic specimen prepared from phloridzin (Cremer and Seuffert, *Ber.*, 1912, **45**, 2566). Since β-*p*-hydroxyphenylpropionic acid yields phenol, *p*-hydroxybenzoic acid, and acetic acid on fusion with potash (Barth, *Annalen*, 1869, **152**, 96; Barth and Schreder, *Ber.*, 1879, **12**, 1259), the presence of traces of phenol and of acetic acid in the crude product was explained.

Dibromoleucodrin.—Leucodrin (6 g.) in methyl alcohol (50 c.c.) was treated in the cold with bromine (6·5 g.) in methyl alcohol (50 c.c.). The mixture became warm, and the methyl alcohol and the slight excess of bromine were then distilled off. The residue crystallised from methyl alcohol in colourless prisms, m. p. 254—255° [Found: C, 37·0, 37·5; H, 3·3, 3·3; Br, 33·2, 33·3; reactive H (Zerewitinow estimation in pyridine), 0·66, 0·67. $C_{15}H_{14}O_8Br_2$ (three reactive hydrogens) requires C, 37·3; H, 3·0; Br, 33·2; reactive H, 0·64%]. *Dibromoleucodrin* was soluble in sodium bicarbonate solution. For titration experiments, it was dissolved in alcohol and titrated with N/20-baryta, back-titration in the hot being used: the titre was three times that required to bring the solutions to neutrality in the cold [Found: equiv., by back-titration, 160. $C_{15}H_{14}O_8Br_2$ (one acidic and two lactone groups) requires equiv., 161].

In quantitative brominations of leucodrin, this substance was dissolved in water, an excess of N/10-potassium bromide–bromate mixture added, and the whole acidified. After 15 minutes the excess of bromine was estimated iodometrically [Found: *M* (assuming dibromination), 323. Calc. for $C_{15}H_{16}O_8$: *M*, 324].

Tetra-acetyl Dibromoleucodrin.—On acetylation with acetic anhydride dibromoleucodrin yielded a solid substance, which crystallised from dilute acetic acid in colourless prisms, m. p. 195—196° [Found: C, 42·4; H, 3·4; Br, 24·5; CO·CH_3, 25·4. $C_{15}H_{10}O_8(CO{\cdot}CH_3)_4$ requires C, 42·4; H, 3·5; Br, 24·5; CO·CH_3, 25·2%], insoluble in sodium bicarbonate solution.

Tetrapropionyl dibromoleucodrin, prepared with propionic anhydride, crystallised from dilute propionic acid or from alcohol in matted needles, m. p. 106—108° [Found: C, 46·2; H, 4·4. $C_{15}H_{10}O_8Br_2(CO{\cdot}CH_2{\cdot}CH_3)_4$ requires C, 45·9; H, 4·3%].

Dichloroleucodrin.—This was formed by the repeated evaporation of a leucodrin solution in the presence of concentrated hydrochloric acid and hydrogen peroxide. It crystallised from water in colourless prisms, m. p. 236·5—239°, and in all its properties resembled the corresponding dibromoleucodrin [Found: C, 46·4, 46·0; H, 3·7, 3·8; Cl, 17·4; equiv., by back titration, 129. $C_{15}H_{14}O_8Cl_2$ (one acidic and two lactone groups) requires C, 45·8; H, 3·6; Cl, 18·0%; equiv., 131].

Tetra-acetyl dichloroleucodrin crystallised from slightly diluted acetic acid in colourless prisms, m. p. 171—172°. Like the corresponding dibromo-compound, it was insoluble in sodium bicarbonate solution, and could not be titrated with alkali in the cold [Found: C, 49·6; H, 4·3; Cl, 12·8; CO·CH_3, 30·5. $C_{15}H_{10}O_8Cl_2(CO{\cdot}CH_3)_4$ requires C, 49·1; H, 4·1; Cl, 12·6; CO·CH_3, 30·1%].

The Action of Nitric Acid on Leucodrin.—To leucodrin (1 g.), moistened with water, was added a mixture of nitric acid (4 c.c.) and water (6 c.c.); the whole became warm and orange-red. The crystals obtained after heating on the water-bath for a few minutes were collected, washed with water, and recrystallised from this solvent. The new substance separated in long, flat, slightly yellow prisms. It dissolved readily in sodium bicarbonate solution and in alkalis to give deep orange solutions. The crystals decomposed slowly above 240°, and melted to a dark liquid at 251° [Found: C, 43·9, 43·7; H, 3·6, 3·7; N, 7·0, 7·0. $C_{15}H_{14}O_8(NO_2)_2$ requires C, 43·5; H, 3·4; N, 6·8%]. It appears as if a *dinitroleucodrin* had been formed. With higher concentrations of nitric acid, oily products resulted.

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THE UNIVERSITY OF CAPE TOWN, SOUTH AFRICA. [*Received, November 12th*, 1937.]