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CONSTITUENTS OF *LEUCADENDRON* SPECIES[1]—I

A CHEMICAL INVESTIGATION OF THE STRUCTURE OF LEUCODRIN

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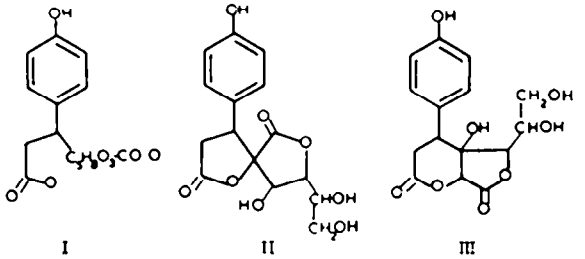
Abstract—The structure of leucodrin, the bitter principle from *Leucadendron adscendens*, has been shown to be II by chemical investigation.

LEUCODRIN, the main constituent of the *Leucadendron* species was isolated as early as 1895[3,4] with little information regarding its chemical constitution. Rapson[5–7] made the first serious attempt to determine the structure when he established that it was a dilactone of molecular formula $C_{15}H_{16}O_8$ which contained one phenolic OH and three alcoholic OH groups. Furthermore, since he isolated 1-anisylsuccinic acid along with one mole formaldehyde from alkaline periodate oxidation of leucodrin monomethyl ether, he concluded that the metabolite contained the partial structure I.



I II III

Although a complete elucidation of the structure of leucodrin was greatly hampered by his failure to obtain tractable products from the majority of degradations, this worker tentatively proposed the two structures (II and III) as best explaining the chemistry of the compound. Nevertheless, neither of these structures seemed

[1] A. W. Murray and R. W. Bradshaw, *Tetrahedron Letters* 3773 (1966).
[2] Present address, Dyson Perrins Laboratory, Oxford. Work performed for Ph.D. at Manchester University, 1964.
[3] E. Merck, *Merck's Ber.* 3 (1895).
[4] O. Hesse, *Liebigs Ann.* 290, 314 (1896).
[5] W. S. Rapson, *J. Chem. Soc.* 282 (1938).
[6] W. S. Rapson, *J. Chem. Soc.* 1085 (1939).
[7] W. S. Rapson, *J. Chem. Soc.* 1271 (1940).

entirely satisfactory. Periodic acid oxidation of the monomethyl ether dihydrate[8] of II would be expected to yield 1-keto-2-anisylglutaric acid and not anisylsuccinic acid. On the other hand, under the same conditions, the monomethyl ether dihydrate of III would be expected to yield glyoxylic acid as one of the products, but even the elegant method of Fosse and Huille[9] repeatedly failed to show the presence of this acid among the products of the oxidation.

Preliminary studies confirmed the above mentioned anomalies and consequently a chemical investigation of the structure of leucodrin was initiated.

Leucodrin, $C_{15}H_{16}O_8$, was isolated in 19% yield by chromatographic separation on powdered 'Perlon'.[10] The UV spectrum of the metabolite strongly supports the presence of a *p*-substituted phenolic chromophore in the molecule.[11] Consonant with this formulation is the observed absorption in the IR at 1620 cm^{-1} which lies well within the normal range expected for a *p*-disubstituted aromatic system. In addition to displaying bands characteristic of three alcoholic OH groups and one phenolic group, leucodrin exhibits strong CO absorption at 1778 and 1803 cm^{-1}. The former band suggests the presence of a γ-lactone system,[12] but the latter at 1803 cm^{-1} may be explained by the presence of any one of the following lactone systems: an $\alpha\beta$-unsaturated γ-lactone;[13] a lactone containing bulky *cis* substituents;[14] a spiro-type δ-lactone[15] or a γ-lactone with an electronegative substituent in the γ-position.[16] Since all attempts (catalytic hydrogenation, epoxidation, addition of bromine, reaction with tetranitromethane) to establish the presence of unsaturation in the molecule, other than benzenoid unsaturation, failed, the first of these arrangements can be precluded. The IR spectrum of leucodrin monomethyl ether dihydrate exhibits no CO absorption other than that of the carboxylate ion and as a result it is reasonable to conclude that each of the carbon atoms in the C_5 residue of I is oxygenated because this residue has to accommodate three OH functions and the points of attachment of the two lactone rings. The parent monomethyl ether was regenerated by acidification of the dihydrate.

Although Rapson had already investigated the periodate oxidation of leucodrin and its derivatives, he failed to obtain degradation products of significant size, other than anisylsuccinic acid, from this reaction. However, in this present investigation it was found that under carefully controlled oxidation conditions, leucodrin consumed one mole of sodium metaperiodate and afforded two degradation products, formaldehyde (one mole) and an aldehydic product, leucodrin noraldehyde, $C_{14}H_{12}O_7$, which showed absorption in the IR at 1720 cm^{-1} (>CHO) and a peak in the NMR at 0·48 τ attributable to an aldehydic proton. This aldehyde was further characterized as its

[8] The term "dihydrate" is used to describe the ring open form of leucodrin and its derivatives in which both lactone rings have been cleaved by the action of base. Similarly, the term "monohydrate" is employed elsewhere to describe a similar rupture of one lactone ring in leucodrin and its derivatives.

[9] R. Fosse and A. Huille, *C.R. Acad. Sci., Paris* **181**, 286 (1925).

[10] A. W. Murray, *Nature, Lond.* 484 (1962).

[11] R. F. Hunter, R. A. Morton and A. T. Carpenter, *J. Chem. Soc.* 441 (1950).

[12] R. B. Woodward and H. Kovach, *J. Am. Chem. Soc.* **75**, 71 (1953).

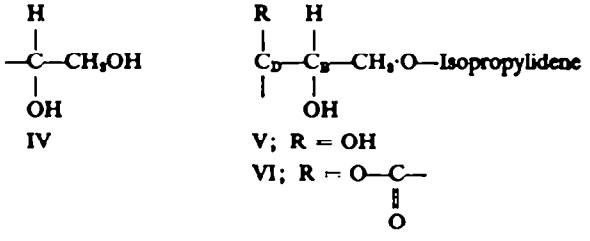
[13] D. H. Whiffen and W. H. Thompson, *J. Chem. Soc.* 1005 (1946).

[14] E. R. H. Jones and J. S. Stephenson, *J. Chem. Soc.* 2197 (1959).

[15] S. Searles, M. Tamres and G. M. Barrow, *J. Am. Chem. Soc.* **75**, 71 (1953).

[16] E. Brügel, K. Dury, G. Stengel and H. Suter, *Angew. Chem.* **68**, 440 (1956).

p-nitrophenylhydrazone, $C_{20}H_{17}N_3O_8$. The production of this compound demands the presence of system IV in leucodrin and this partial skeleton was extended by investigating the periodate oxidation of isopropylidene leucodrin monomethyl ether, which derivative was prepared by Rapson[5] who reported that its ring-open form did not react with periodate. In the present work this was not found to be the case; the isopropylidene derivative reacted with alkaline periodate with the consumption of one mole of reagent. Subsequent removal of the isopropylidene grouping yielded a product which registered positive tests with aldehyde reagents. The fact that this product gave an orange colouration with citric acid,[17] while treatment with α-naphthol and conc sulphuric acid produced a violet colour on heating,[18] immediately suggested the presence of glycolaldehyde in the reaction mixture. That this was indeed the case was confirmed by the isolation of the *p*-nitrophenylosazone of glycolaldehyde.



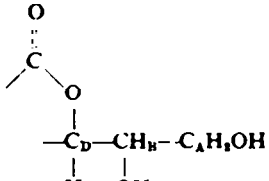
Since leucodrin contains but one —CHOH—CH_2OH grouping this function must be the one involved in the generation of glycolaldehyde, and further, the aldehydic function in this fragment can only have arisen from periodate cleavage of a free vicinal diol of the type V. Periodate titrations indicated that isopropylidene leucodrin itself has no oxidizable glycol grouping, consequently, the OH function at C_D in the above structure must originate from ring opening of one of the lactone rings. The lactonic form of V can therefore be represented as VI.

As well as being of prime importance for structural elucidations, the detection of glycolaldehyde in this reaction also enables the structure of isopropylidene leucodrin to be established. There are only three alcoholic functions in leucodrin, two of which must be utilized in the formation of this derivative, but the OH groups at C_A and C_B cannot be involved together, otherwise alkaline periodate followed by acid hydrolysis would generate not glycolaldehyde, but a C_3 or larger fragment. Moreover, the alcoholic moiety at C_B and the remaining OH group cannot be involved since such a formulation would not be expected to consume periodate in the ring-open form, particularly since it was shown that leucodrin tetramethyl ether dihydrate does not react with periodate. The isopropylidene derivative must therefore involve the primary OH group and the one remaining alcoholic function whose position has yet to be established.

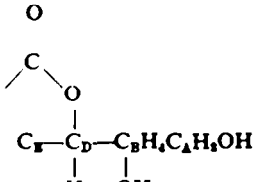
Partial structure VI received confirmation from the following results: Leucodrin noraldehyde was readily reduced by sodium borohydride to the corresponding noralcohol, $C_{14}H_{16}O_7$, which was shown to be a primary alcohol by virtue of the fact that although it remained unaffected by sodium metaperiodate, treatment with alkaline periodate caused evolution of one mole formaldehyde. This result demonstrates that C_D must contain a potential OH function. Leucodrin noralcohol formed

[17] J. B. Shoesmith, C. E. Sosson and A. C. Hetherington, *J. Chem. Soc.* 2223 (1927).
[18] E. Molish, *Monat. Fur. Chem.* 7, 198 (1870).

a trityl derivative which on treatment with alkaline periodate furnished glycolaldehyde trityl ether, isolated as its *p*-nitrophenylhydrazone which was shown to be identical with an authentic specimen. Such a result signifies that the oxygenated function adjacent to the primary alcohol group in leucodrin noralcohol (viz. C_D) must be the site of a hydrogen atom as in VII. This deduction was corroborated by the oxidation



VII

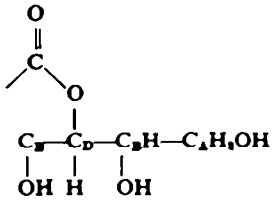


VIII

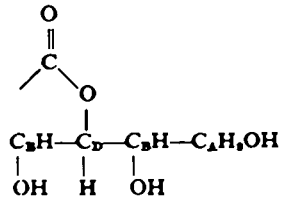
of leucodrin noraldehyde with Fehling's solution to the corresponding carboxylic acid, $C_{14}H_{12}O_8$ (ν_{max} 1703 cm^{-1}) which on treatment with alkaline periodate furnished glyoxylic acid, identified by its colour reaction with pyrogallol carboxylic acid[19] and by preparation of its phenylhydrazone, which gave an undepressed m.p. when mixed with an authentic sample.

Leucodrin monomethyl ether underwent a similar sequence of reactions. For example it readily afforded the corresponding aldehyde, leucodrin monomethyl ether noraldehyde, which compound, on methylation, yielded a dimethyl ether and on borohydride reduction furnished the corresponding noralcohol which on base treatment and subsequent periodate oxidation, consumed one mole of oxidant and generated one mole formaldehyde.

Returning to partial structure VII, C_D must be the site of one other carbon atom in order that leucodrin does not break down irreversibly on treatment with base. Moreover, this carbon atom, C_E in structure VIII, must be attached to an oxygen atom (for reasons mentioned earlier) but not the ether oxygen of the second lactone system, a conclusion drawn from the observation that completely hydrated leucodrin tetramethyl ether does not consume periodate; in other words, that the free OH groups generated on opening the lactone rings of leucodrin tetramethyl ether are not on adjacent carbon atoms. Hence the partial skeleton for leucodrin now becomes IX.



IX



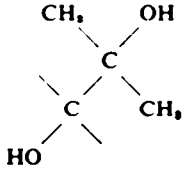
X

Chemical proof that C_E is the site of a secondary alcohol function followed from treatment of leucodrin monomethyl ether noralcohol with one mole of base and one mole of periodate. Cleavage of the second lactone ring with more base and subsequent reduction of the carbonyl reaction products with borohydride, afforded glycerol, detected by VPC. The presence of this entity was confirmed by comparing

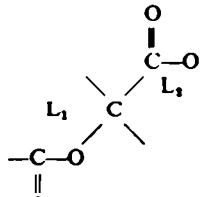
[19] E. Eegriwe, *Zent. Anal. Chem.* **100**, 34 (1935).

the IR spectrum of the polyacetate of the reaction product with that of an authentic specimen of glycerol triacetate. Such a result is only consistent with C_E being the site of a secondary alcohol grouping and the partial structure for leucodrin being X.

Treatment of leucodrin tetramethyl ether with methyl Grignard afforded useful information regarding the remainder of the leucodrin skeleton. In this reaction base was added after excess reagent had been destroyed in order to ensure that both lactone rings were opened. The fact that the product at this stage consumed one mole of periodate, which reagent has no action on leucodrin tetramethyl ether dihydrate, can only be explained by the cleavage of a diol which is generated under the conditions of the reaction. Moreover, acetone isolated as its 2,4-dinitrophenylhydrazone, was evolved in 44% yield, a clear indication that the diol produced in this reaction must have the skeleton XI, where from it can be deduced that the original dilactone must contain the partial structure XII

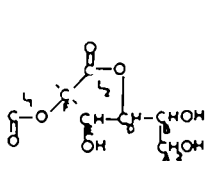


XI

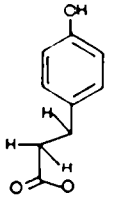


XII

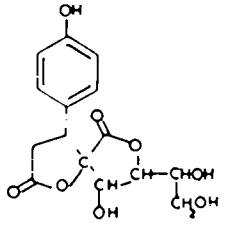
It has already been established that a lactone ring must be situated at C_D in X and as there are only two lactonic functions in the molecule it must be either L_1 or L_2 in XII Lactone L_1 is clearly not linked to C_D since this particular carbon atom is also the site of a hydrogen atom and the two secondary alcoholic groups, C_B and C_E. Therefore lactone L_2 must be attached to C_D and leucodrin can be assigned the partial structure XIII.



XIII



XIV



XV

The earlier experiments of Rapson, which were confirmed in the present investigation, established that the moiety XIV is present in leucodrin. The only way this function can be included in XIII is as shown in XV. In this formula all the atoms of leucodrin have been included and the only possible way in which the remaining carbon atoms can be linked together is to give II as the structure of leucodrin.

The assignment of structure II to leucodrin confirms the results of the X-ray analysis of dibromoleucodrin by Rogers and Diamond[20] and the NMR studies of Perold and Pachler[21] which were published about the same time as structure II was deduced in these laboratories.

[20] D. Rogers and D. Diamond, *Proc. Chem. Soc.* 63 (1964).
[21] G. W. Perold and K. Pachler, *Proc. Chem. Soc.* 62 (1964).

EXPERIMENTAL

Microanalyses were carried out by Mr. V. Manokin and Drs. F. and E. Pascher.

M.ps were taken on a Kofler hot-stage apparatus and are uncorrected. IR spectra determined on Perkin–Elmer 21 and Infracord spectrophotometers, were measured for Nujol mulls unless otherwise stated. UV spectra were determined for EtOH solns with a Hilger Uvispeck spectrophotometer (model 700,305) and, except where otherwise stated ^{1}H NMR spectra were determined at 60 Mc/s with a Varian A-60 spectrophotometer using TMS as an internal reference. Mass spectral data were obtained on an A.E.I. mass spectrophotometer.

Chromatographic separation

Dried, powdered *leucadendron adscendens* leaves (50 g), in a Soxhlet thimble, were extracted for 48 hr with boiling EtOH (1 l.), the EtOH removed *in vacuo* and the resulting dark green viscous syrup dissolved in water (120 ml). The resulting soln was poured on to a column of powdered 'Perlon' (Versuchsprodukt PP15/Sp). The column was first washed with cold water until the effluent was clear and then eluted with boiling water until the eluent gave no colour with $FeCl_3$aq. Concentration of the resulting eluent *in vacuo* yielded leucodrin (9·5 g, 19% based on the dry wt of the leaf).

Leucodrin

Leucodrin, after repeated crystallization from water formed elongated prisms, m.p. 212–212·5° (lit[8] 212–212·5°). (Found: C, 55·4; H, 4·9. Calc. for $C_{16}H_{16}O_8$ = C, 55·5; H, 5·0%), $[\alpha]_D^{26}$ −19·45° (*c*, 0·47 in EtOH). The soln used in this determination was treated with 1N NaOH, allowed to stand for 10 min, and then acidified with H_2SO_4aq, when it showed immediately $[\alpha]_D^{26}$ +30·7° (*c*, 0·215); after 60 hr a steady value of $[\alpha]_D^{26}$ −17·1° was observed. ν_{max} (KBr disc) 3672, 3562, 3370, 3210, 1803, 1778, 1620, 1459, 1412, 1352, 1319, 1263, 1232, 1192, 1116, 1064, 1055 and 832 cm^{-1}; λ_{max} 205 mμ (ε, 7150), 230 mμ (ε, 9660) and 277 mμ (ε, 1430). ^{1}H NMR spectrum (in D_2O) showed signals at 2·54, 2·69, 2·98, 3·11 (q), 5·12, 5·32, 5·63 (t), 6·07, 6·18 (d), 6·36, 6·47 (d), 6·69, 6·76, 6·87 and 6·94 τ (d of d). A mass spectrum showed no molecular ion, but gave peaks at *m/e* 192, 162, 149, 134, 119, 105, 91, 85, 65, 57, 55, 44, 30 and 29. An ORD curve in MeOH gave peaks at 220 ([φ] −35,080), 243 ([φ] +11,200, 268 ([φ] +2600) and 277 mμ ([φ] −1100).

0·0957N NaOH (0·21 ml) was added to leucodrin (22·5 mg) and the sample heated *in vacuo* at 105° for 2 hr. Acidification of the resulting Na salt with 1 drop HClaq gradually caused the separation of *leucodrin monohydrate* as colourless needles, m.p. 92°. (Found = C, 52·8; H, 5·1. $C_{16}H_{16}O_8$ requires: C, 52·6; H, 5·3%); ν_{max} 1778 cm^{-1}.

In a similar manner, by addition of NaOHaq to the Na salt of the monohydrate and heating the solution for 2 hr *in vacuo* at 100°, the Na salt of leucodrin dihydrate was isolated, ν_{max} 1615 and 1583 cm^{-1}. This product was acidified with HClaq and the soln left at room temp for 1 week after which time the solvent was removed *in vacuo*. Trituration of the residual material with EtOH, filtration and removal of the solvent yielded leucodrin.

A 10 mg/ml soln of leucodrin in water was injected intraperitoneally into 4 mice; 2 mice received 50 mg/kg and 2 received 100 mg/kg. These doses had no apparent effects apart from causing slight initial excitement and all 4 mice were found to be apparently normal after 1 week.

Periodate oxidation of leucodrin

A soln of leucodrin (1·3 g) in water (10 ml) was cooled and sodium metaperiodate (870 mg) added when an exothermic reaction took place and the soln turned brown in colour. Gradually *leucodrin noraldehyde* (800 mg) separated as brown needles, which after repeated crystallization from water gave colourless needles, m.p. 178–179°. (Found: C, 54·1; H, 4·6. $C_{14}H_{16}O_7 \cdot H_2O$ requires: C, 54·2; H, 4·5%); ν_{max} 3500, 3300, 1800, 1768, 1720, 1620 and 1350 cm^{-1}; τ (DMSO) C*H*O 0·48 (1H, s), aromatic protons 2·71, 2·87, 3·20, 3·33 (4H, A_2B_2 spectrum), CHO*H* 3·50, 3·60 (1H, d), phenolic O*H* 3·72, 3·81, 3·91 (1H, t), CH_2·CH 5·16, 5·49, 5·69 (3H, ABC spectrum), C*H*·CHO 6·61, 6·68, 6·75, 6·81 (1H, q) and C*H*OH 7·1, 7·2 (1H, d).

An aqueous soln of the product gave a strong positive reaction with Fehling's soln. All attempts to prepare the 2,4-dinitrophenylhydrazone failed, but the *p-nitrophenyl hydrazone* was readily obtained as an orange ppt by mixing aqueous solns of the noraldehyde and *p*-nitrophenylhydrazine sulphate. The derivative was purified by chromatography on alumina by elution with MeOH and

had m.p. 193–195° after crystallization from MeOH. (Found: C, 54·3; H, 4·1; N, 10·1. $C_{20}H_{17}N_5O_6$ requires: C, 54·2; H, 4·0; N, 10·0%.)

The formaldehyde evolved in this oxidation was estimated as its dimedone derivative. (Found: 0·99 mole formaldehyde.)

Leucodrin noraldehyde (50 mg) was dissolved in water (2 ml) and sodium metaperiodate (35 mg) added. After 2 hr unchanged leucodrin noraldehyde was deposited.

Periodate oxidation of the dihydrate of isopropylidene leucodrin monomethyl ether

Isopropylidene leucodrin monomethyl ether (13 mg)[8] which was unaffected by treatment with excess sodium metaperiodate soln, was dissolved in 0·00957N NaOH (9·01 ml) and the soln warmed on a water bath for 2 hr. After cooling, excess sodium metaperiodate soln was added, the mixture left to stand at room temp for 2 hr after which time the periodate consumed was estimated iodimetrically (Found: 0·94 moles sodium metaperiodate consumed).

In a second experiment, isopropylidene leucodrin monomethyl ether (331 mg) was dissolved in 0·957N NaOH (1·82 ml) and the soln heated on a water bath at 100° for 2 hr. On cooling, sodium metaperiodate (177 mg) was added, the mixture left to stand for a further 2 hr after which time it was acidified with 2N HCl and boiled for a final 30 min. The soln was then neutralized to litmus with Na_2CO_3aq. A small portion of this soln on treatment with 10% aq citric acid produced an orange colouration while addition of 20% ethanolic α-naphthol and one drop conc H_2SO_4 to an equal volume of the neutral reaction mixture gave a purple grey colouration which became intense violet on heating. The remainder of the neutral reaction mixture was then evaporated to dryness *in vacuo*. Extraction of the residual solid with EtOH, and removal of the solvent, yielded a small amount of oil, which on dissolution in water (2 ml) and treatment with excess *p*-nitrophenylhydrazine sulphate soln, yielded the osazone of glycolaldehyde (53 mg) identical in all respects with an authentic sample of the *p*-nitrophenylosazone of glycolaldehyde.

Oxidations of leucodrin noraldehyde with Fehling's solution

Leucodrin noraldehyde (100 mg) was dissolved in warm water (3 ml) and freshly prepared Fehling's solution added dropwise until a slight excess of the reagent remained on boiling the soln. The cuprous oxide was removed by filtration and the soln cooled to 0°, when conc HCl was added until the mixture was acidic to litmus. On standing overnight at 0° colourless needles of leucodrin noracid (76 mg) were deposited and crystallized from water, m.p. 242–245° with slight decomposition occurring at 170° and gas being evolved at 248°. (Found: C, 54·5; H, 3·7. $C_{14}H_{12}O_8$ requires: C, 54·5; H, 3·9%); ν_{max} 3280, 1805, 1752, 1703 and 1620 cm^{-1}.

Leucodrin noracid (30 mg) was dissolved in 40% NaOHaq (0·2 ml) and warmed to 75° for 2 hr when sodium metaperiodate (25 mg) was added. After standing for a further 2 hr, positive reactions for glyoxylic acid were obtained using the Eegriwe,[19] Calkins[33] and Fiegel[34] tests. Negative reactions were obtained on blanks. The remaining soln furnished a phenylhydrazone, m.p. 144–146° alone, and when admixed with an authentic sample of glyoxylic acid phenylhydrazone.

Reduction of leucodrin noraldehyde with sodium borohydride

Leucodrin noraldehyde (500 mg) was dissolved in EtOH (5 ml), the soln cooled to −5°, and sodium borohydride (18·5 mg) added in small quantities. The resulting soln was allowed to stand for 12 hr at 0°, after which time excess borohydride was destroyed by the addition of 1 drop of glacial AcOH, and the solvent removed *in vacuo*. The residual pale yellow oil on treatment with water (2 ml) yielded *leucodrin noralcohol* as a cream coloured solid (473 mg) which gave colourless needles, m.p. 223–225° from water. (Found: C, 57·4; H, 5·0. $C_{14}H_{14}O_7$ requires: C, 57·1; H, 4·8%); ν_{max} 3400, 3300, 3110, 1800, 1765 and 1620 cm^{-1}. The noralcohol was unaffected by excess sodium metaperiodate soln, but when the compound (100 mg) in water (5 ml) was treated with 0·0957N NaOH (7·99 ml) and the soln left to stand under N_2 for 24 hr after which time sodium metaperiodate (232 mg) was added, formaldehyde, 0·8 mole, was generated (estimated as its dimedone derivative).

[33] V. P. Calkins, *Ind. Eng. Chem.* (Anal. edit.) **15**, 762 (1943).
[34] F. Feigel, *Spot Tests in Organic Analysis*, Elsevier, 352 (1956).

On heating the noralcohol (45 mg) with Analar pyridine (2 ml) and trityl chloride (40 mg) at 50° for 10 days and pouring the clean soln which resulted into 5N HCl *leucodrin noralcohol trityl ether* (8 mg) was deposited as a pale brown amorphous solid.

Periodate oxidation of leucodrin noralcohol trityl ether

The crude trityl ether of leucodrin noralcohol (30 mg) in 2N NaOH (0·5 ml) was warmed to 70° for 2 hr. After cooling to room temp, sodium metaperiodate (40 mg) was added, the soln allowed to stand for a further 2 hr and then extracted with chf (2 × 25 ml). Removal of the organic solvent afforded a semi-solid product, which was dissolved in aqueous EtOH and treated with *p*-nitrophenylhydrazine solution. On standing, an orange ppt was deposited. This was chromatographed on alumina using chf as eluent. Removal of the chf afforded dark orange needles m.p. >310° alone and undepressed on admixing with an authentic sample of glycolaldehyde trityl ether *p*-nitrophenylhydrazone prepared by periodate oxidation of (±) glyceraldehyde trityl ether and subsequent treatment with *p*-nitrophenylhydrazine. (Found: C, 74·0; H, 5·4; N, 9·6. Calc. for $C_{27}H_{23}N_3O_3$: C, 74·2; H, 5·3; N, 9·6%.)

Periodate oxidation of leucodrin monomethyl ether

Leucodrin monomethyl ether (1·5 g)[4] in water (10 ml) was treated with sodium metaperiodate (900 mg) and after the evolution of formaldehyde ceased (2 hr), *leucodrin methyl ether noraldehyde* separated as an oil. The aldehyde yielded a p-*nitrophenylhydrazone* as bright orange needles, which derivate, after repeated crystallization from aqueous EtOH had m.p. 186–188°. (Found: N, 9·5. $C_{21}H_{19}N_3O_6$ requires: N, 9·5%); ν_{max} 1640 cm^{-1}. *Leucodrin dimethyl ether noraldehyde*, m.p. 48–50° was readily obtained by methylating the monomethyl ether in acetone with methyl iodide and freshly prepared Ag_2O.

Reduction of leucodrin monomethyl ether with sodium borohydride

Leucodrin monomethyl ether noraldehyde (1·05 g) was dissolved in EtOH (7 ml), the soln cooled to 0° and $NaBH_4$ (145 mg) added portionwise. The resulting soln was allowed to stand at the same temp for 12 hr, after which time excess borohydride was destroyed (addition of glacial AcOH) and the solvent removed *in vacuo*. Trituration of the residual pale yellow oil with water yielded solid *leucodrin monomethyl ether noralcohol* (670 mg) which crystallized from water as colourless needles, m.p. 144–146°. (Found: C, 56·3; H, 5·3. $C_{16}H_{16}O_7 \cdot \frac{1}{2}H_2O$ requires: C, 56·8; H, 5·4%); ν_{max} 3110, 1803, 1776 and 1620 cm^{-1}.

A soln of this alcohol (14 mg) in 0·00957N NaOH (6·49 ml) was left to stand at room temp under N_2 for 24 hr, after which time excess sodium metaperiodate soln was added and the soln allowed to stand for a further 2 hr when the periodate consumed was estimated iodometrically (Found: 1·12 moles sodium metaperiodate consumed).

To determine the amount of formaldehyde generated in this reaction, the monomethylated noralcohol (76 mg) was dissolved in 0·0957N NaOH (7·04 ml), the soln warmed on a water bath for 2 hr, cooled, and sodium metaperiodate (238 mg) added. After a further 2 hr sat. AcONaaq (5 ml) was added followed by a 5% soln of dimedone in EtOH (5 ml). The soln was shaken at frequent intervals during 3 hr and the formaldehyde dimedone derivative (91 mg) which was collected, had m.p. 189° alone and when mixed with an authentic specimen. (Found: 0·94 mole formaldehyde.)

Periodate oxidation of the monohydrate of leucodrin monomethyl ether noralcohol

Leucodrin monomethyl ether noralcohol (100 mg) was dissolved in 0·0957N NaOH (0·799 ml) and the soln allowed to stand at room temp for 24 hr, when sodium metaperiodate (750 mg) was added. After 2 hr the soln was cooled to 0° $NaHB_4$ (50 mg) added in small portions and the mixture left to stand for a further 2 hr. The soln was basified with NaOHaq, left to stand at room temp for 24 hr and evaporated to dryness. Extraction of the residual paste with EtOH (2 × 25 ml), and removal of the solvent *in vacuo* afforded an oil (21 mg) which was characterized by GLC on silicone at 300°, as glycerol, by comparison of column retention times with an authentic sample.

In a second run the glycerol was converted to its triacetate by treatment with Ac_2O (2 ml) and a trace of $ZnCl_2$, at 180° for 3 hr, when on removal of the solvent the residual oil distilled at 256–258. (lit[84] b.p. 258–259°) ν_{max} 2940, 2900, 1745, 1422, 1368, 1339, 1240–1175 (broad), 930, 871 and 772 cm^{-1}.[10]

[84] W. H. Perkin and J. L. Simonsen, *J. Chem. Soc.* **87**, 858 (1905).

Leucodrin tetramethyl ether

Leucodrin (500 mg) was methylated with diazomethane according to Rapson's procedure.[8] The anhydrous monomethyl ether was dissolved in Analar acetone (2·5 ml) and redistilled MeI (50 ml) added. Freshly prepared Ag_2O (10 g) was added and the mixture shaken continuously for 24 hr after which time the Ag_2O was filtered off and the MeI removed. The methylation was repeated 5 times using fresh Ag_2O and fresh MeI each time. After the sixth methylation the acetone was removed and the residual yellow solid (110 mg) crystallized 3 times from EtOH from which it separated as needles, m.p. 125–126° (lit[8] 125–126°). (Found: C, 59·8; H, 6·3; OMe, 32·4. Calc. for $C_{19}H_{34}O_8$: C, 59·9; H, 6·3; OMe, 32·6%.)

Dissolution of leucodrin tetramethyl ether in 0·2N NaOH and treatment of the cooled soln with excess sodium metaperiodate showed no consumption of the oxidant.

Reaction of leucodrin tetramethyl ether with methyl Grignard

Leucodrin tetramethyl ether (100 mg) was dissolved in re-distilled, Na-dried ether (1·51) and MeMgI (from 1 g Mg) added to the mixture which was refluxed for 2 hr. The resulting Mg complex was destroyed with 1N HCl (50 ml) and the ether removed by distillation. The remaining aqueous soln was divided into 2 equal portions. To one portion was added sodium metaperiodate (100 mg), the soln boiled in a stream of N_2 and the effluent gas passed into aqueous 2,4-dinitrophenylhydrazine soln. After 3 hr no solid had separated. The remaining 25 ml of soln was treated with excess 2N NaOH, filtered, and the filtrate heated at 70° for 30 min, after which time sodium metaperiodate (100 mg) was added. The passage of N_2 through the aqueous reaction mixture and the passage of the effluent gas through aqueous 2,4-dinitrophenylhydrazine resulted in the precipitation of acetone 2,4-dinitrophenylhydrazone (62·7 mg, 44%) m.p. and mixed m.p. 156–158°.

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