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CONSTITUENTS OF *LEUCADENDRON* SPECIES—II

THE CHEMISTRY OF LEUCODRIN

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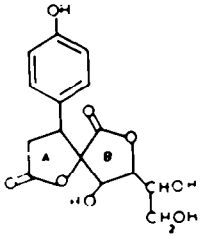
Abstract—Attempts are made to explain the chemistry of leucodrin on the basis of its structure I.

PREVIOUS work by physical[2,3] and by chemical[4] methods has unequivocally shown that the structure of leucodrin is I. Nevertheless, during our chemical investigation of this metabolite there arose an interesting observation which was not readily susceptible to rationalization on the basis of structure I. Namely, since model reactions with



periodate established that at room temperature, 1-ketoacids consume negligible amounts of periodate, one would expect that leucodrin monomethyl ether, if based on structure I would yield, on treatment with alkali and periodate, 1-keto-2-anisylglutaric acid and not the observed anisylsuccinic acid.

The explanation we have invoked to explain this anomalous result is a straightforward hemiketalization in solution (not without precedent)[5–8] involving the primary alcohol function of the leucodrin molecule as shown in II. Under alkaline conditions only lactone A would ring open immediately and the resulting vicinal triol would, on periodate cleavage, give rise to anisylsuccinic acid and a sugar carbonate as shown

[1] Present address, Dryson Perrins Laboratory, Oxford. Work done for Ph.D. Manchester University, 1964.
[2] D. Rogers and D. Diamand, *Proc. Chem. Soc.* 63 (1964).
[3] G. W. Perold and K. Pachler, *Proc. Chem. Soc.* 62 (1964).
[4] A. W. Murray and R. W. Bradshaw, *Tetrahedron* Part I (1966).
[5] W. N. Haworth and E. C. Hirst, *J. Chem. Soc.* 1045 (1927).
[6] H. Adkins and A. E. Broderick, *J. Am. Chem. Soc.* **50**, 499 (1928).
[7] M. L. Wolfrow, *Ibid.* **52**, 2464 (1930); **53**, 2275 (1931); **54**, 3390 (1932).
[8] A. R. Peacocke and J. B. Smith, *Chem. & Ind.* 1383 (1957).

in Fig. 1. Some indication that one of the lactone rings (ring A) in leucodrin and its derivatives is much more susceptible to base attack than the second lactone ring, was gleaned from treatment of leucodrin monomethyl ether noralcohol with one mole of alkali and sodium metaperodate, when 9·1% of the available formaldehyde (estimated as its 2,4-dinitrophenylhydrazone) was released.

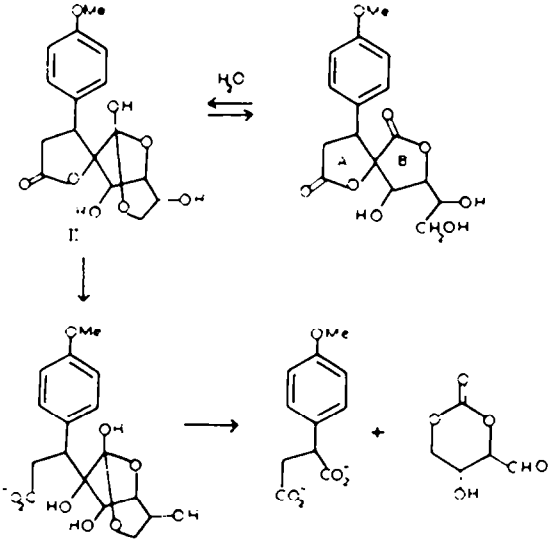


FIG. 1

The liberation of carbon dioxide under the oxidation conditions used by Rapson[9] can then be explained by breakdown of this carbonate in the following manner (Fig. 2).

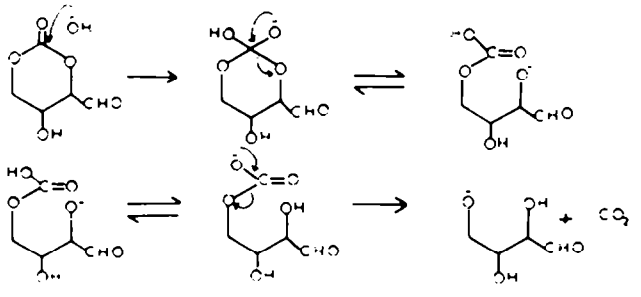
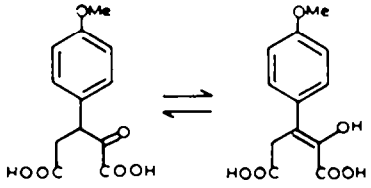


FIG. 2

If such a hemiketalization does occur then the course of this reaction may be affected by extrusion of the primary alcohol residue from the parent compound. Consequently the monomethyl ether of leucodrin noraldehyde, which compound dissolves in base but is regenerated unchanged on acidification, was subjected to alkaline periodate. The product of the reaction exhibited absorption in the IR at 1783 and 1725 cm^{-1}, very similar to that displayed by 1-ketoglutaric acid, while its UV spectrum, which was very similar to that of leucodrin monomethyl ether, showed an aromatic K band at 228 mμ (ε, 12,500) significantly more intense than the corresponding band

[9] W. S. Rapson, *J. Chem. Soc.* 1271 (1940).

for that compound (ϵ, 9400), a result indicative of the presence of a partially extended chromophore such as would exist in the enol form of 1-keto-2-anisylglutaric acid (III). The constitution of this degradation product was finally put beyond all doubt by



III

comparison of its isonitroso- and *p*-nitrophenylhydrazone derivatives with authentic samples prepared by the action of amyl nitrite on 1-carbethoxy-2-anisyl diethylglutarate[10] and the Japp–Klingeman reaction[11,12] of *p*-nitrophenylhydrazine sulphate on 1-carbethoxy-2-anisyl diethylglutarate, respectively, as shown in Fig. 3.

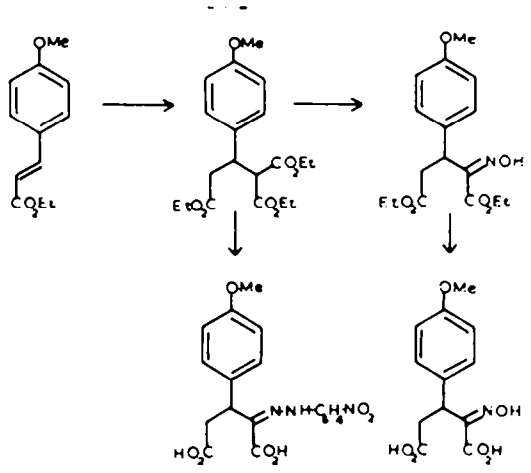


FIG. 3

It is interesting to note here that on using Rapson's conditions for periodate oxidation of leucodrin monomethyl ether dihydrate, carbon dioxide (one mole) is evolved, as theoretically predicted by the mechanism presented in this paper. On the other hand, when care is taken to ensure that both lactone rings are cleaved, no carbon dioxide should be evolved, and experimentally this is indeed found to be the case.

Support for these deductions was forthcoming from the results of periodate treatment of dinitroleucodrin bishydroxydiamide (the dinitro derivative was used instead of the parent bishydroxydiamide because the latter compound furnished intractable oils under the conditions of the oxidation). In this compound, $C_{15}H_{20}N_4O_{12}$, the formation of the hemiketal is no longer feasible and the expected product 1-keto-2-

[10] C. H. Harington, *J. Biol. Chem.* **64**, 34 (1925).
[11] F. R. Japp and F. Klingeman, *Ber.* **20**, 2942, 3284 (1887).
[12] R. P. Linstead and A. B. Wang, *J. Chem. Soc.* 807 (1937).

(3,5-dinitro-4-hydroxy)phenylglutaramide, $C_{11}H_{10}N_4O_8$, (ν_{max} 1708, 1674 and 1648 cm^{-1}) is obtained on its periodate oxidation.

The results presented in this paper therefore permit an explanation for the anomalous behaviour of leucodrin on alkaline-periodate treatment.

EXPERIMENTAL

Microanalyses were carried out by Mr. V. Manokin and by 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, ^1H 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.

Periodate oxidation of leucodrin

Leucodrin (50·7 mg) was dissolved in 1N NaOH (5·0 ml) and excess 0·0131N sodium metaperiodate (45 ml) added. After 2 hr the CO_2 evolved was estimated gravimetrically as $BaCO_3$. (Found: 0·8 mole CO_2 evolved.) In a second run, leucodrin (32·6 mg) was dissolved in 1N NaOH (10 ml) and the soln left to stand for 24 hr. Excess 0·0131N sodium metaperiodate (30 ml) was added and after 2 hr the CO_2 evolved was estimated. (Found: 0·0 mole CO_2 evolved.)

In these reactions a blank was performed on the same volume of reagents and the purity of the $BaCO_3$ was determined by adding excess standard HCl, and back-titrating the acid with standard NaOH.

Periodate oxidation of leucodrin monomethyl ether

Leucodrin monomethyl ether (1·5 g) was dissolved in 1N NaOH (12 ml) and a soln of sodium metaperiodate in excess of dil H_2SO_4 added. After 2 hr the soln was saturated with $(NH_4)_2SO_4$ and extracted with ether. The ether extracts were washed with $Na_2S_2O_3$aq, dried and the ether removed. The residual anisylsuccinic acid crystallized from aqueous alcohol and had m.p. 197–199° (lit[13] 194–195). On treatment with acetyl chloride the acid furnished an anhydride, m.p. 91° (lit[13] 90·5°).

Periodate oxidation of the dihydrate of leucodrin monomethyl ether noraldehyde

Leucodrin monomethyl ether noraldehyde (900 mg) was dissolved in 0·957N NaOH (6 ml) and the soln allowed to stand for 24 hr under N_2. Sodium metaperiodate (1·8 g) in water (10 ml) and Analar perchloric acid (1 ml) was added and the mixture left at room temp for a further 2 hr. On cooling to 0°, a pale cream solid (225 mg) was deposited; concentration of the mother liquor afforded a further crop of crystals (141 mg). The combined product was collected, washed with ice-water (2 ml), dried and crystallized from acetonitrile when it has m.p. 190–192°. (Found: C, 57·0; H, 4·9. $C_{13}H_{13}O_6$ requires: C, 57·2; H, 4·8%); ν_{max} 3450, 3100, 2700-2400, 1783, 1725 and 1620 cm^{-1}, λ_{max} 228 mμ (ε, 12500), 266–268 mμ (inflexion) (ε, 1300–1400), 276 mμ (ε, 1900), 282 mμ (ε, 1700) and 294–303 mμ (ε, 310–260). The ^1H NMR (DMSO) showed signals at τ 2·77, 2·91, 3·11, 3·27 (A_2B_2 spectrum), 3·8–4·2 (ABC spectrum), 5·37(s), 6·07(s) and 6·39(s). Treatment of the product with aqueous thiosemicarbazide and AcONa afforded the *thiosemicarbazone* as colourless plates, m.p. 194–196°, radiating needles at 150°, while reaction with $NH_2OH.HCl$aq in the presence of AcONa yielded *1-isonitroso-2-anisylglutaric acid*, m.p. 145–146°, alone and when mixed with a synthetic sample. The *p-nitrophenylhydrazone* had m.p. 195–197° which remained undepressed on admixture with a synthetic sample of *1-keto-2-anisylglutaric acid* *p-nitrophenylhydrazone*.

1-Isonitroso-2-anisylglutaric acid

p-Methoxy ethyl cinnamate[14] (25 g) was condensed with diethylmalonate. (20·5 g) in the presence of Na (340 mg) at 100° for 2 hr and then at 150° for a further 1½ hr. After cooling to 0° the resulting brown oil was stirred with 2N H_2SO_4 (100 ml) and the mixture extracted with ether (3 × 50 ml).

[13] W. Baker and A. Lapworth, *J. Chem. Soc.*, **127**, 560 (1925).

[14] K. C. Pandya, *J. Ind. Chem. Soc.* **11**, 823 (1934).

The combined organic fractions were washed with water (2 × 50 ml), dried and the ether removed. Distillation of the residual oil afforded *1-carbethoxy-2-anisyl diethylglutarate* (23·4 g) which came over at b.p. 216–218°/0·4 mm. (Found: C, 61·8; H, 7·5. $C_{18}H_{26}O_7$ requires: C, 62·2; H, 7·1%); ν_{max} (liquid film) 2950, 2890, 2805, 1750, 1728 and 1620 cm^{-1}.

1-Carbethoxy-2-anisyl diethylglutarate (10 g) was dissolved in amyl nitrite (4·2 g) at 0°, the soln added slowly and with stirring to Na (670 mg) in abs EtOH (17 ml) and the mixture allowed to stand overnight at −5°. The alcohol was removed in a vacuum desiccator over conc H_2SO_4, the residual dark brown oil dissolved in water (10 ml) and the resulting soln saturated with CO_2. After extraction with ether (2 × 25 ml) and removal of the solvent, *1-isonitroso-2-anisyl-diethylglutarate* (4·1 g) was deposited. The ester was partially purified by repeated dissolution in 2N NaOH (10 ml) and reprecipitation with CO_2, the purified ester (3 g) dissolved in a soln of KOH (6 g) in water (5 ml) and the mixture warmed to 50° for 10 min and then at 100° for 5 min. Acidification with 2N HCl extraction with ether (3 × 25 ml) and removal of the solvent, afforded a brown oil which on trituration with warm benzene yielded *1-isonitroso-2-anisylglutaric acid* as a cream coloured solid (1·07 g). The acid crystallized from acetonitrile as pale cream prisms, m.p. 145–146° (Found: C, 53·9; H, 5·0; N, 5·2. $C_{13}H_{13}NO_6$ requires: C, 54·0; H, 4·9; N, 5·2%); ν_{max} 3400, 3030, 2920, 2700–2200, 1702, 1668, 1630 and 1620 cm^{-1}.

1-Keto-2-anisylglutaric acid p-*nitrophenylhydrazone*

1-Carbethoxy-2-anisyl diethylglutarate (10 g) was dissolved in the minimum quantity of 25% aqueous EtOH at −5° and a soln of NaOH (3·5 g) in water (15 ml) added to the mixture with stirring. *p*-Nitroaniline (3·78 g) in conc HCl (3 ml) and water (20 ml) was then added over a period of 15 min. The soln was stirred for ½ hr at 0°, acidified to litmus with 5N HCl and the dark brown oil which deposited, was cooled to 0°, when it gradually solidified. Repeated crystallization from aqueous EtOH afforded orange-red prisms of *1-keto-2-anisylglutaric acid* p-*nitrophenylhydrazone*, m.p. 195–197°, (Found: C, 55·6, H. 4·4; N, 10·8. $C_{18}H_{17}N_3O_7$ requires: C, 55·9; H, 4·4; N, 10·9%); ν_{max} 3580, 3515, 3200, 3030, 2700–2100, 1700, 1669 and 1615 cm^{-1}.

Periodate oxidation of leucodrin bishydroxydiamide

Leucodrin bixhydroxydiamide (900 mg), prepared by the procedure of Rapson[16] was dissolved in cold water (15 ml) and sodium metaperiodate (4 g) in water (15 ml) added, when a dark brown intractable oil (460 mg) slowly separated.

Dinitroleucodrin bixhydroxydiamide

Dinitroleucodrin[16] (2·5 g) was dissolved in the minimum quantity of EtOH (150 ml), the soln cooled to 0° and saturated with ammonia gas. The resulting deep orange soln was left to stand in the refrigerator for 24 hr when an amorphous powder (1·26 g) was deposited; concentration of the mother liquor *in vacuo* yielded a further crop of solid (1·33 g). A small portion of *dinitroleucodrin bishydroxydiamide* was crystallized from EtOH but it decomposed with evolution of ammonia, so no further attempt was made to purify the material which had m.p. 178–179°, ν_{max} 3460, 3345–3120, 2740–2390, 1670, 1625, 1235 and 1048 cm^{-1}.

Periodate oxidation of dinitroleucodrin bishydroxydiamide

The bishydroxydiamide of dinitroleucodrin (2·5 g), dissolved in water (12 ml) was treated with sodium metaperiodate and the resulting exothermic reaction controlled by cooling the orange coloured reaction mixture in an ice bath. The mixture was allowed to stand at room temp for 24 hr and the yellow solid which separated, was thoroughly extracted with boiling EtOH (6 × 10 ml). Evaporation of the alcoholic extracts *in vacuo* afforded *1-keto-2-(3,5-dinitro-4-hydroxy) phenylglutaramide* as a amorphous orange powder (1·2 g) which crystallized from water as long yellow needles, m.p. 146–148°. (Found: C, 38·7; H, 3·4; N, 16·1. $C_{11}H_{10}N_4O_8$ requires: C, 38·4; H, 3·4; N 16·2%); ν_{max} (CaF_2 prism) 3520, 3430, 3350, 3300, 1708, 1700, 1674, 1669, 1646, and 1605 cm^{-1}, λ_{max} 210 mμ (ε, 20300), 234 mμ (inflexion) (ε, 13200), 250 mμ (ε, 2800) and 440 mμ (ε, 1480).

The above phenylglutaramide (100 mg) was treated with 20% NaOH (5 ml) and the resulting

[16] W. S. Rapson, *J. Chem. Soc.* 284 (1938).

orange soln warmed to 60° in a stream of N_2. The effluent gases were bubbled through 2 traps each containing standard H_2SO_4 (25 ml) for 2 hr and the ammonia evolved in the hydrolysis was estimated by back titration. (Found: 1·8 mole ammonia evolved.)

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