The Senecio Alkaloids. Structure and Absolute Configuration of Swazine

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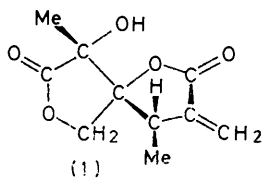
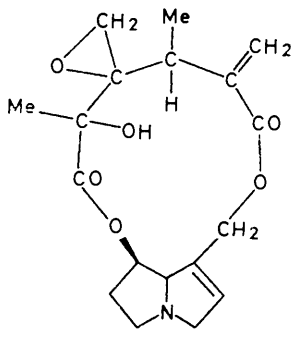
The structure and conformation of swazine (3), a pyrrolizidine alkaloid from *Senecio swaziensis* Compton, is determined from chemical and spectral data.

In a preliminary publication [1] the new pyrrolizidine alkaloid swazine, $C_{18}H_{23}NO_6$, m.p. 165°, $[\alpha]_D$ −103·5° (EtOH), $\lambda_{max.}$ (H_2O) 195 nm (ε 11,000), was reported together with retrorsine; first from plants of *Senecio swaziensis* Compton, collected at Mbabane, Swaziland, and later from plants of *S. barbellatus* D.C., collected near Wartburg, South Africa.

Swazine yielded, on acid hydrolysis, the known base retronecine and the spiro-dilactone (1), $C_{10}H_{12}O_5$, m.p. 191° $[\alpha]_D$ −116·6° (EtOH), $\lambda_{max.}$ (H_2O) 213 nm (ε 9700), c.d. Δε −8·19 (213 nm). Structure (2) was previously proposed for swazine. We now present the full chemical and physical evidence for (1) and show that swazine must have the overall structure (3) not (2), and in addition the conformation shown in (3) is suggested for swazine.



(1)



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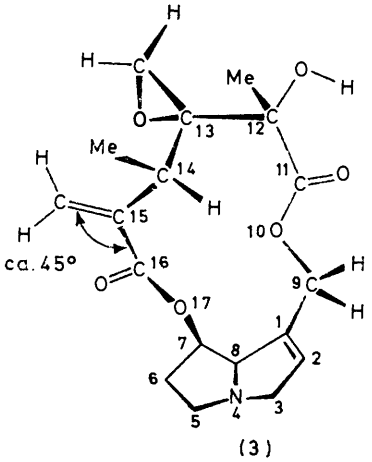


(3)

Titration of (1) with base at room temperature and back titration after refluxing with the excess of base showed the presence of two lactone rings of different stability. Furthermore (1) showed a u.v. maximum at 213 nm (ε 9700) indicative of αβ-unsaturation, and readily afforded a mono-acetate and a mono-3,5-dinitrobenzoate. This evidence, taken with the n.m.r. spectrum of (1)

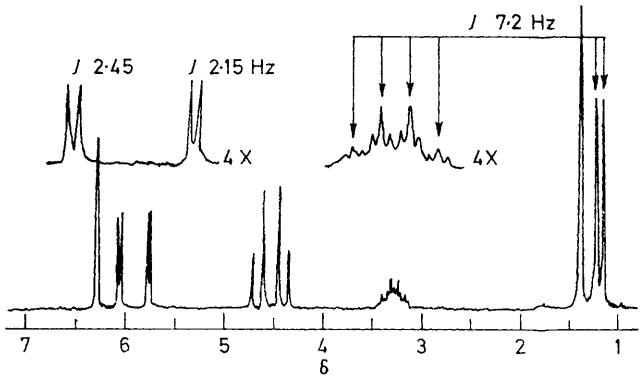
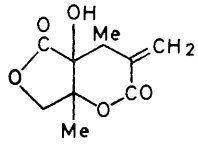


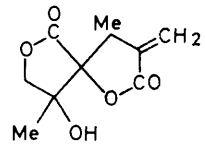
FIGURE 1 N.m.r. spectrum of the spiro-dilactone [100 MHz; $(CD_3)_2SO$]

(Figure 1), was consistent with two possible acid structures each of which could be lactonised in two ways. The first possibility was a glutaric acid of the trichodesmic type [(4a) or (4b)], so far found only from plants of the family Boraginaceae,[2,3] and the second possibility, an adipic acid of the sceleranecic type [(5a) or (5b)], found only in *S. sceleratus*.[4] Precedent favoured the latter.

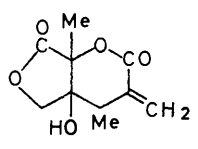
In the mass spectrum of (1) (Scheme 1) the prominent loss of the fragment $C_3H_4O_3$ favoured (5b) over structures (4a) and (4b). Goulden and Manning [5] have shown that in α-hydroxy-acids with the general formula $R^1R^2C(OR^3)CO_2R^4$, the base peak is due to the ion $R^1R^2CO^+R^3$. If this step is applicable to lactones then the fragments



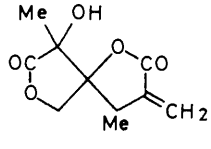
(4a)



(4b)



(5a)



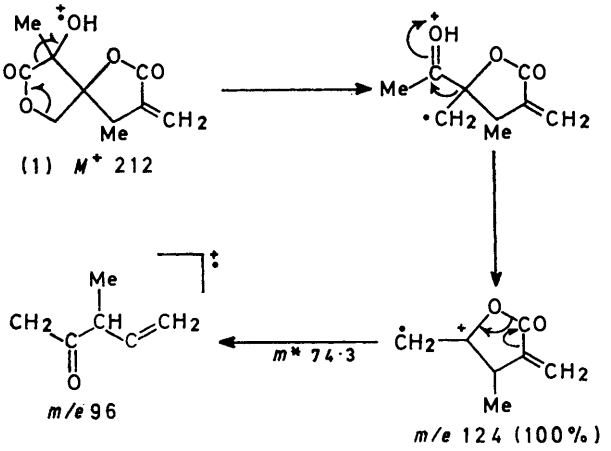
(5b)

m/e 124 and 96 could arise from (5b) as shown in Scheme 1.

Catalytic hydrogenation of (1) gave the dihydro-dilactone, $C_{10}H_{14}O_5$, m.p. 203°, with lactone rings of different

[1] C. G. Gordon-Gray, R. B. Wells, N. Hallak, M. B. Hursthouse, S. Neidle, and T. P. Toube, *Tetrahedron Letters*, 1972, 707.
[2] F. L. Warren, *Fortschr. Chem. Org. Naturstoffe*, 1966, 24.
[3] L. B. Bull, C. C. J. Culvenor, and A. T. Dick, 'The Pyrrolizidine Alkaloids, Their Chemistry, Pathogenecity, and Other Biological Properties,' North-Holland Publishing Co., Amsterdam, 1968.
[4] H. L. de Waal, A. Wiechers, and F. L. Warren, *J. Chem. Soc.*, 1963, 953.
[5] J. D. S. Goulden and D. J. Manning, *Org. Mass Spectrometry*, 1970, **3**, 1467.

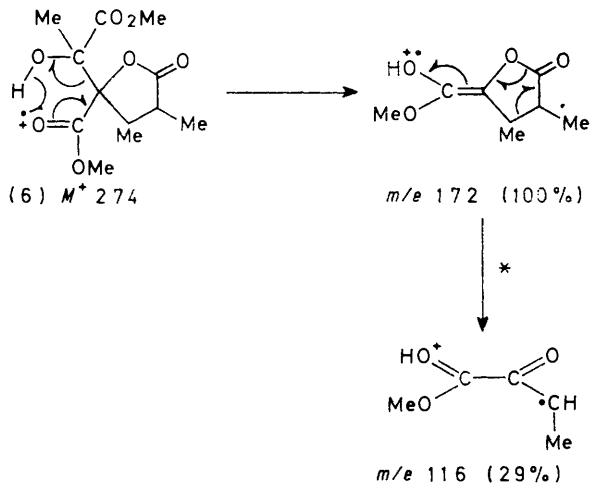
stability towards base. In the n.m.r. spectrum a doublet methyl signal at δ 1·14 replaced the doublets at 5·76 (1H) and 6·06 (1H), ascribed to an exocyclic methylene group in (1).



SCHEME 1

Oxidation with 1% aqueous permanganate, under conditions sufficiently basic to open only one lactone ring, gave a dibasic α-hydroxy-acid, $C_{10}H_{14}O_7$, m.p. 193°, in 65% yield. Comparison of the n.m.r. spectra of the dihydro-dilactone, the hydroxy-acid, and its dimethyl ester (6) clearly showed that the hydroxymethyl group (AB q, δ 4·52), exposed by opening ring A, had been oxidised to a carboxy-group. The mass spectrum of (6) (Scheme 2) showed a prominent loss of a fragment mass 102, to give an ion, *m/e* 172, which then further decayed to give *m/e* 116.

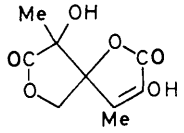
The α-hydroxy-acid was cleaved with an excess of sodium bismuthate to give (−)-2,3-dimethylsuccinic



SCHEME 2

acid, carbon dioxide (1·8 mol), and acetic acid (0·8 mol). After purification by vacuum sublimation and repeated recrystallisations, the acid compared satisfactorily with known (−)-2,3-dimethylsuccinic acid by i.r., n.m.r., m.p., and rotation, and hence C-4 has the *S*-configuration.[6] Traces of the *meso*-form may have been lost during purification but the catalytic reduction appears to have been largely stereospecific.

Ozonolysis of (1) at −70° gave formaldehyde and a compound (7), $C_9H_{10}O_6$, m.p. 205°. I.r. bands at 3270 (OH, broad strong), and 1695 cm^{-1} (sharp) indicated that (7) contained an acidic hydroxy and an olefinic link. Tests[7] showed that (7) was enolic and the n.m.r. spectrum showed no trace of the α-keto-compound. All the signals in the n.m.r. spectrum of (7) were unsplit, a peak at δ 10·13 being ascribed to the group C=C–OH.



(7)

The u.v. spectrum of (7) in water agreed with spectra of similar compounds reported by Berner and Kolsaker.[8,9]

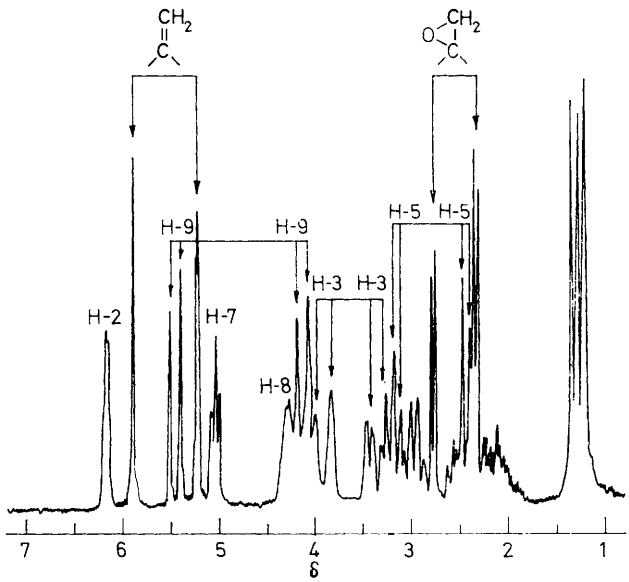


FIGURE 2 N.m.r. spectrum of swazine (100 MHz; $CDCl_3$)

Methanolysis of (1) using a catalytic amount of potassium methoxide in methanol produced no trans-esterification,[10] but instead the addition compound (8), $C_{11}H_{16}O_6$, m.p. 173°, was formed. An attempt to transesterify (1) using hydrogen chloride in methanol also failed, unchanged (1) being recovered.

The molecular formula of swazine and the presence of a 1,2,3-triol system in (1) suggested that swazine might contain an exocyclic epoxide at position 3 of the acid portion. The n.m.r. spectrum of swazine (Figure 2) showed two one-proton doublets at δ 2·77 and 2·31 (*J* 4 Hz), and these signals were readily decoupled and

[6] G. E. McCasland and S. Proskow, *J. Amer. Chem. Soc.*, 1956, **78**, 5646.

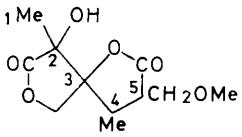
[7] N. D. Cheronis and J. B. Entrikin, 'Semimicro Qualitative Organic Analysis,' Interscience, New York, 1957, 2nd edn., pp. 224 and 228.

[8] E. Berner and P. Kolsaker, *Acta Chem. Scand.*, 1969, **23**, 597.

[9] F. Mo and B. K. Sivertsen, *Acta Cryst.*, 1971, **27**, 115.

[10] M. Reimer and H. R. Downes, *J. Amer. Chem. Soc.*, 1921 945.

agreed well with patterns reported for terminal epoxides.[11,12]



(8)

Prolonged hydrolysis of swazine with an excess of barium hydroxide gave retronecine, an uncharacterised gum, and in 40% yield a monobasic α-hydroxy-acid (9), $C_{10}H_{14}O_6$, m.p. 200°, named isoswazinecic acid. Hydrolysis of swazine for shorter times or with less base gave a mixture of gum, acid (9), and dilactone (1). Hydrolysis under mild conditions using IRA 400 resin in the basic form at 45° gave only (1). These results indicated that the dilactone (1) was the initial product of base hydrolysis and that isoswazinecic acid (9) resulted from the further action of base on (1). This was proved when nearly equal amounts of isoswazinecic acid (9) and the acidic gum were obtained in overall 90% yield by boiling (1) for 18 h in a large excess of aqueous barium hydroxide.

The methyl ester of isoswazinecic acid showed i.r. absorption bands at 3520 (OH), 1790 (γ-lactone), and 1740 cm^{-1} (ester). The formula of (9) and the absence of the band at 1625 cm^{-1}, typical of the αβ-olefin, suggested that a six-membered ether ring had been formed by nucleophilic attack of the hydroxymethyl group onto the αβ-olefin. This structure was supported by the n.m.r. spectrum, the two one-proton doublets due to the exocyclic methylene being replaced by a two-proton doublet at δ 3·90 coupled (J 2 Hz) to a one-proton signal at δ 2·58 which was in turn coupled to a one-proton multiplet at δ 3·23 ($J_{4,5}$ 5·2, $J_{4,7}$ 7 Hz).

The formation of the ether ring in isoswazinecic acid by direct attack of the hydroxymethyl group onto the αβ-olefin could occur under conditions sufficiently basic to keep both lactone rings open (Figure 3).

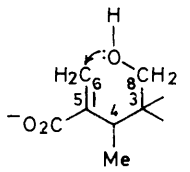
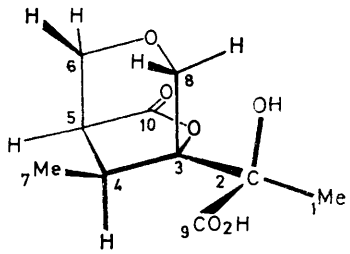


FIGURE 3

Examples of this type of reaction under both acidic and basic conditions have been reported by Shoppee and Hughes.[13] Protonation of the carbanion which develops on C-5 can occur from either side; isolation of two products, the gum and (9), in equal amounts indicated that there was no preference for either epimer. The acidic gum was possibly the C-5 stereoisomer for which closure of the γ-lactone on final acidification was impossible. C-4 Was known to have the *S*-configuration and closure of the lactone required either a (3*S*,5*R*)- or (3*R*,5*S*)-configuration. Models showed that in the first case the dihedral angle between the protons on C-4 and C-5 was *ca.* 80° and in the second *ca.* 40°. Decoupling showed $J_{4,5}$ to be 5·2 Hz, strongly favouring the configuration (3*R*,4*S*,5*S*) as opposed to (3*S*,4*S*,5*R*) for which $J_{4,5}$ should be nearly zero. From the *X*-ray analysis [1] (1) has the configuration (2*R*,3*R*,4*S*), and hence isoswazinecic acid confirms the configuration of C-3 and has the configuration (2*R*,3*R*,4*S*,5*S*).

Culvenor converted the epoxy-alkaloids jacobine and jacozine into the olefinic analogues, senecionine and seneciphylline respectively, by using potassium selenocyanate.[14] Extended treatment of swazine with this reagent failed to release red selenium which normally signals olefin formation *via* the episelenide.



(9)

The position of the ester links between swazinecic acid and the base retronecine, shown in structure (3), was established by catalytic hydrogenolysis of swazine. When a dilute solution of swazine in ethanol containing a trace of ammonia was hydrogenated over Adams catalyst 3 mol. equiv. of hydrogen were absorbed. The product (10), which precipitated during reduction, was a powder, $C_{18}H_{29}NO_6$, m.p. 183° (decomp.). The high solubility of (10) in water, and i.r. bands at 3400 (OH), 2340 (internal salt), 1730 (ester), and 1600 cm^{-1} (carboxy-zwitterion) showed that it was a zwitterion formed by hydrogenolysis of the allylic ester, leaving the secondary ester intact.[15,16] A solution of (10) in dilute sulphuric acid was rapidly extracted with ether which failed to remove any material from the aqueous layer, proving that the acidic and basic portions were still linked. Zwitterion (10) gave a positive test for an α-hydroxy-acid, proving that the carboxy-unit of this group had been linked in the ester to the allylic hydroxy-group of retronecine.

Comparison of the n.m.r. spectrum of (10) with that of swazine showed changes consistent with reduction of the terminal methylene, the allylic ester, and the double bond in the base. The epoxide was not reduced and in spite of overlap with other signals the epoxy doublets were clear and readily decoupled.

The half-ester zwitterions formed from retronecine diesters vary in their stability towards base and acid; often they cannot be isolated because internal transesterification cleaves the secondary ester.[3] When

[11] 'High Resolution N.M.R. Spectra Catalog,' Varian Associates, Palo Alto, 1962, p. 32.

[12] S. M. Kupchan, J. E. Kelsey, M. Maruyama, J. M. Cassady, J. C. Hemingway, and J. R. Knox, *J. Org. Chem.*, 1969, **34**, 3876.

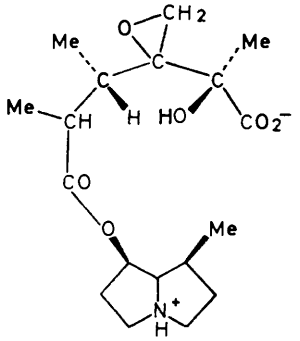
[13] C. W. Shoppee and N. W. Hughes, *J. Chem. Soc.* (*C*), 1971, 3673.

[14] C. C. J. Culvenor, *Austral. J. Chem.*, 1964, **17**, 233.

[15] R. Adams and E. F. Rogers, *J. Amer. Chem. Soc.*, 1941, **63**, 537.

[16] R. Schoental, *Austral. J. Chem.*, 1963, **16**, 233.

swazine was hydrogenated in dilute perchloric acid over Adams catalyst 3 mol. equiv. of hydrogen were consumed and the acid system was completely cleaved from the retronecanol. The gummy acidic product was a



(10)

mixture (t.l.c.). Neutral reduction in ethanol gave a mixture of (10) and a gum, but these complications were avoided by reduction under mildly basic conditions.

Acidic hydrolysis of (10) gave retronecanol and a neutral compound, m.p. 154°, with the same empirical formula ($C_{10}H_{14}O_5$) as the dihydro-spiro-dilactone, m.p. 203°. The i.r. spectra of these compounds showed only slight differences in the fingerprint region and their mass spectra were virtually identical, indicating that they were C-5 diastereoisomers. Models of the dihydro-dilactone indicated dihedral angles between H-4 and H-5 of *ca.* 90° for the absolute configuration (4*S*,5*S*), and 30° for (4*S*,5*R*), these angles being consistent with the observed coupling constants of $J_{4,5}$ 0, and $J_{4,5}$ 12 Hz respectively.

Reduction of swazine to yield the 5*R*-configuration in (10) must reflect adsorption on the catalyst in the opposite sense to the spiro-dilactone, but no conclusion as to the conformation of swazine can be drawn because fission of the allylic ester may precede reduction of the exocyclic methylene.

The foregoing results established the gross structure and the absolute stereochemistry of swazine but did not explain the absence of an absorption maximum above 200 nm in the u.v. spectrum; distortion of the normally planar αβ-unsaturated ester chromophore might account for this.

The possibility of crowding and strain in the macrocyclic ring of pyrrolizidine diesters has been noted in several alkaloids lacking αβ-unsaturation.[17-19] From the conformational point of view structure (3) differed in two ways from alkaloids of the senecionine type; namely the rigidity and size of the epoxy-substituent on C-13, and the presence of a methyl group on C-14. Using the approach adopted by Culvenor *et al.*[19] the probable conformation of swazine was deduced by comparing the n.m.r. spectrum of swazine with those of known alkaloids, in particular jacobine, known by *X*-ray analysis.[20] Conformational restraints thus deduced were imposed on a model of swazine to test the effects of crowding.

The width (9 Hz) of the H-7 multiplet of swazine indicated normal buckling of *ca.* 40° in the saturated ring of retronecine, while the chemical shift of this multiplet (δ 5·05) indicated that the ester carbonyl was roughly *cis*-coplanar with H-7, as in jacobine. The widely spaced H-9 doublets (δ 4·12, 5·48) indicated the conformation usual for the allylic ester in adipic diesters of retronecine, *i.e.* with one H-9 lying near the intersection of the ester and the olefin planes. These features were imposed on the model; ester groups were kept planar and the α-hydroxy-group was kept near the ester plane to allow for the expected hydrogen bonding.[21] This model showed that the *trans*-coplanar conformation of the αβ-unsaturated ester chromophore was most unfavourable, forcing the other ring substituents to lie in one plane along the outer edge of the diester ring and requiring an impossibly close approach of the epoxy-oxygen to the ester oxygens. When the epoxy-oxygen, the C-16 carbonyl, and the oxygen at position 10 were roughly equidistant (*ca.* 3·5 Å) the ring substituent adopted a staggered conformation with the exocyclic methylene at an angle of *ca.* 45° to the ester plane and *cis* to the carbonyl [see (3)]. In this conformation the C(14)–H bond lay near the plane of the exocyclic methylene, in good agreement with the barely detectable allyic coupling.

The reasoning used here to establish the structure and conformation of swazine is referred to in Laing's [22] subsequent work on the solid methiodide in which he used *X*-ray crystallography to confirm the foregoing conclusions.

EXPERIMENTAL

Extraction of Swazine (3).—Dried, ground plant material (2·5 kg) of *S. swaziensis* Compton was extracted with ethanol in the usual way.[23] The crude alkaloid in dry ethyl acetate (50 ml) deposited retrorsine (10 g) on standing. The mother liquor was chromatographed on alumina (Merck, activity II—III) in ethyl acetate. *Swazine*, (13,13-epoxymethano-12-hydroxy-12,14-dimethyl-15-methylenecrotal-1-enine) * (3) was eluted first, and crystallised from acetone (charcoal) as shiny needles (23 g, 0·9%), m.p. 165° (Found: C, 61·9; H, 6·8. $C_{18}H_{23}NO_6$ requires C, 61·9; H, 6·7%) (tar formation prevented satisfactory nitrogen analysis), $[\alpha]_D^{20}$ −104° (*c* 1·16 in ethanol), $\nu_{max.}$ ($CHCl_3$) 3520, 1720, 1637, 1268, and 1160 cm^{-1}, $\lambda_{max.}$ (H_2O) 195 nm (ε 11,000), *m/e* 349·151 ($C_{18}H_{23}NO_6$ requires 349·153), 305·162 ($C_{17}H_{23}NO_4$ requires 305·163), 288·159 ($C_{17}H_{22}NO_3$ requires 288·160), and 262·144 ($C_{15}H_{20}NO_3$ requires 262·144).

Methiodide of Swazine.—Swazine (500 mg), treated with methyl iodide (1 ml) in methanol (50 ml) for 24 h, gave the *methiodide* (450 mg), m.p. (from methanol) 202—205°

* This name is based on the system of nomenclature proposed in ref. 19.

[17] M. P. Cava, K. V. Rao, J. A. Weisbach, R. F. Raffauf, and B. Douglas, *J. Org. Chem.*, 1968, **33**, 3570.
[18] D. H. G. Grout, *J. Chem. Soc.* (*C*), 1969, 1379.
[19] C. C. J. Culvenor, D. H. G. Grout, W. Klyne, W. P. Mose, J. D. Renwick, and P. M. Scopes, *J. Chem. Soc.* (*C*), 1971, 3653.
[20] J. Fridrichsons, A. M. Mathieson, and D. J. Sutor, *Acta Cryst.*, 1963, **16**, 1075.
[21] C. C. J. Culvenor and R. Dal Bon, *Austral. J. Chem.*, 1964, **17**, 1296.
[22] M. Laing and P. Sommerville, *Tetrahedron Letters*, 1972, 5183.
[23] M. J. Koekemoer and F. L. Warren, *J. Chem. Soc.*, 1951, 66.

(decomp.) (Found: C, 46·6; H, 5·5; I, 26·0; N, 2·7. $C_{19}H_{26}INO_6$ requires C, 46·4; H, 5·3; I, 25·8; N, 2·9%).

Acidic Hydrolysis of Swazine.—Swazine (3) (13 g) and 3N-sulphuric acid (200 ml) were heated under reflux for 16 h. Continuous extraction of the cooled solution with chloroform gave (4S,5R,6R)-6-*hydroxy*-4,6-*dimethyl*-3-*methylene*-1,8-*dioxaspiro*[4.4]*nonane*-2,7-*dione* (1), which crystallised from acetone–chloroform (charcoal) as long needles (6·1 g), m.p. 191°, $[\alpha]_D^{20}$ −117° (*c* 1·63 in ethanol), c.d. (H_2O) $\Delta\varepsilon$ −8·19 (213 nm) (Found: C, 56·4; H, 5·9; C–Me, 1·84%; equiv., 214 and 113. $C_{10}H_{12}O_5$ requires C, 56·6; H, 5·7; C–Me, 2·0%; equiv., 212 and 106), $\nu_{max.}$ (KBr) 3420, 1782, 1771, 1740sh, and 1662 cm^{-1}, $\nu_{max.}$ ($CHCl_3$) 3570, 1798, 1788, 1740sh, and 1665 cm^{-1}, $\lambda_{max.}$ (H_2O) 213 nm (ε 9700), *m/e* 212·069 ($C_{10}H_{12}O_5$ requires 212·068), 141·055 ($C_7H_9O_3$ requires 141·055), 124·052 ($C_7H_8O_2$ requires 124·052), 95·049 (C_6H_7O requires 95·050), 82·042 (C_5H_6O requires 82·042), and 54·047 (C_4H_6 requires 54·047). The hydrolysate was made alkaline, taken to dryness, and extracted with ether to give a solid, which, on crystallisation from dry ether, gave retronecine, m.p. 121° (Found: C, 61·9; H, 8·7. Calc. for $C_8H_{13}NO_2$: C, 61·9; H, 8·4%).

Acetylation of the Spiro-dilactone (1).—Dilactone (1) (100 mg), pyridine (1 ml), and acetic anhydride (1 ml) were heated at 100° for 4 h. The mixture was poured into cold sodium hydrogen carbonate solution, taken to dryness under vacuum, and extracted with ether. The *acetate*, recrystallised from acetone–cyclohexane, gave plates (80 mg), m.p. 99° (Found: C, 56·5; H, 5·3. $C_{12}H_{14}O_6$ requires C, 56·7; H, 5·6%).

Dilactone 3,5-*Dinitrobenzoate*.—The dilactone (1) (100 mg), 3,5-dinitrobenzoyl chloride (200 mg), and pyridine (1 ml) were heated under reflux (30 min). The mixture was poured into cold potassium carbonate solution, and the crude ester was filtered off, washed with 2N-sulphuric acid, and crystallised from acetone–chloroform (charcoal) as prisms, m.p. 235° (Found: C, 50·5; H, 3·7. $C_{17}H_{14}N_2O_{10}$ requires C, 50·3; H, 3·5%).

Ozonolysis of the Dilactone (1).—The dilactone (1) (300 mg) in ethyl acetate was ozonised at −70°. The ozonide was decomposed with water and steam-distilled to give formaldehyde, precipitated as its dimedone derivative. Extraction of the non-steam volatile residue with ether gave a solid which was crystallised from acetone–chloroform to give 3,6-*dihydroxy*-4,6-*dimethyl*-1,8-*dioxaspiro*[4.4]*non*-3-*ene*-2,7-*dione* (7) as clusters of plates (130 mg), m.p. 205° (Found: C, 50·5; H, 4·9%; equiv., 218. $C_9H_{10}O_6$ requires C, 50·5; H, 4·7%; equiv., 214), $\nu_{max.}$ (KBr) 3270, 1780, 1740, and 1696 cm^{-1}, $\lambda_{max.}$ (H_2O) 243 nm (ε 7900), shoulder at 275 nm, *m/e* 214 (M^+, 1%), 196 (4), 168 (14), 153 (7), 127 (14), 126 (100), 83 (15), and 81 (31), δ [60 MHz; $(CD_3)_2SO$] 1·42 (3H, s, C–Me), 1·93 (3H, s, C=C–Me), 4·51 (2H, ABq, C–CH_2O), 6·17 (1H, s, OH), and 10·13 (1H, s, C=C–OH). Chemical tests [7] showed that compound (7) was enolic. Enol (7), treated with diazomethane, and sublimed under vacuum, gave the crystalline methyl ether, m.p. 191°, *m/e* 228 (M^+, 4%), 141 (43), 140 (100), 127 (16), 111 (63), 98 (19), and 83 (69).

Catalytic Hydrogenation of the Dilactone (1).—The dilactone (1) (1 g), in ethanol (300 ml) over Adams catalyst (100 mg) absorbed 127 ml of hydrogen (0·95 mol) at 21° and 707 mmHg. The product was recrystallised from ethanol to give 6-*hydroxy*-3,4,6-*trimethyl*-1,8-*dioxaspiro*[4.4]*nonane*-2,7-*dione* as cubic crystals, m.p. 203° (Found: C, 55·8; H, 6·5. $C_{10}H_{14}O_5$ requires C, 56·1; H, 6·6%), $\nu_{max.}$ (KBr) 3400, 1780, and 1755 cm^{-1}, $\nu_{max.}$ ($CHCl_3$) 3570 and 1796 cm^{-1}, *m/e* 214 (M^+, 5%), 126 (47), 111 (19), 98 (44), 72 (28), and 56 (100).

Oxidation of the Dihydro-dilactone.—The foregoing dihydro-dilactone (isomer, m.p. 203°) (1·55 g) in water (50 ml) at *ca.* 90° was titrated with 2N-potassium hydroxide to the first end point (phenolphthalein), and a second equivalent of alkali was added at 20°. Potassium permanganate solution (1%) was added over 2 h until the permanganate colour persisted. After dissolving the manganese dioxide (Na_2SO_3–conc. H_2SO_4) the solution was concentrated (50 ml), and continuously extracted with ether to give gummy crystals (1·3 g). The acidic product was recrystallised from dry acetone–chloroform to give 2,3-*dihydroxy*-4-*methylhexane*-2,3,5-*tricarboxylic acid* γ-*lactone* as plates (1·2 g), m.p. 194° (Found: C, 48·6; H, 5·7%; equiv., 134. $C_{10}H_{14}O_7$ requires C, 48·8; H, 5·7%; equiv., 123), $\nu_{max.}$ (KBr) 3460, 2980, 1780, 1730, and 1670 cm^{-1}. The foregoing acid (100 mg) in tetrahydrofuran (10 ml) was treated with an excess of ethereal diazomethane. After removing the solvent the solid was sublimed (70° at 0·05 mmHg), and crystallised from acetone–light petroleum (b.p. 40—60°) to give the *dimethyl ester* (6) as thin flakes, m.p. 93°, *m/e* 274 (M^+, 2%), 215 (85), 172 (100), 171 (26), 155 (87), 143 (33), 116 (29), 115 (25), and 59 (50).

Bismuthate Oxidation of the Foregoing Acid.—Sodium bismuthate (12 g) was added over 48 h to a stirred solution of the acid (1·86 g) (obtained by the permanganate oxidation above) in 3·3M-phosphoric acid (60 ml) and water (100 ml). The filtered solution was continuously extracted with ether, and much aqueous phase was carried over. This was separated, the ether was shaken out with a little water, and the combined aqueous fractions were re-extracted with ether. The combined ether extracts gave a gummy crystalline acid (450 mg), which, after crystallisation from acetone–benzene, sublimation (70° at 0·005 mmHg), and recrystallisation from methanol–benzene, gave (−)-2,3-dimethylsuccinic acid (216 mg) as long needles, m.p. 132° (softening at 128°), $[\alpha]_D^{20}$ −8·5° (*c* 0·735 in H_2O) (lit., $[\alpha]_D^{20}$ −8°, m.p. 135°) (Found: C, 49·4; H, 6·9. Calc. for $C_6H_{10}O_4$: C, 49·3; H, 6·9%). The volatile products of the above oxidation were determined quantitatively on a micro-scale. Sodium bismuthate (800 mg) in water (8 ml) and 3·3M-phosphoric acid (3 ml) was swept out with nitrogen to remove traces of carbon dioxide. After adding the hydroxy-acid (70 mg, 0·29 mmol) the evolved carbon dioxide was trapped as barium carbonate (122 mg, 0·62 mmol). Steam-distillation of the oxidation mixture in a C–Me apparatus gave acetic acid (0·23 mmol), identified by the i.r. spectrum of the barium salt.

Attempted Base-catalysed Transmethylation of the Dilactone (1).—The dilactone (1) (1 g) and potassium (10 mg) in absolute methanol (100 ml) were refluxed under nitrogen for 48 h. The solution was neutralised with 2N-sulphuric acid, diluted with water (100 ml), and concentrated to 20 ml. Continuous extraction with ether gave a solid (1·07 g), which was crystallised from acetone–benzene to give 6-*hydroxy*-3-*methoxymethyl*-4,6-*dimethyl*-1,8-*dioxaspiro*[4.4]-*nonane*-2,7-*dione* (8) as cubic crystals, m.p. 171—173° (Found: C, 54·0; H, 6·6; OMe, 12·1. $C_{11}H_{16}O_6$ requires C, 54·1; H, 6·6; OMe, 12·7%), $\nu_{max.}$ (KBr) 3300 and 1780 cm^{-1}, *m/e* 244 (M^+, 8%), 156 (21), 124 (36), 111 (100), and 82 (47). In a similar experiment the 4-*ethoxymethyl-compound* was obtained, m.p. 176° (Found: C, 55·9; H, 7·1. $C_{12}H_{18}O_6$ requires C, 55·8; H, 7·0%).

Alkaline Hydrolysis of Swazine (3).—Swazine (3) (4 g) and barium hydroxide (16 g) in water (500 ml) were refluxed for 15 h. The solution was acidified with 2N-sulphuric acid, centrifuged, concentrated (50 ml), and then continuously extracted with chloroform to give a gummy product (2·23 g). The crude product was dissolved in chloroform and extracted with 2N-sodium carbonate solution; evaporation of the chloroform solution left a trace of neutral gum. The carbonate solution was acidified and then extracted with chloroform to give (2R)-2-*hydroxy*-2-{(1R,5S,8S)-8-*methyl*-6-*oxo*-3,7-*dioxabicyclo*[3.2.1]*octan*-1-*yl*}*propionic acid* (*isoswazinecic acid*) (9) (1·08 g), m.p. 200° (from acetone–chloroform). Isoswazinecic acid (9) softened at 190° and bubbled strongly after melting but was sublimed without decomposition at 120° and 0·05 mmHg (Found: C, 52·2; H, 6·1. $C_{10}H_{14}O_6$ requires C, 52·2; H, 6·1%), $\nu_{max.}$ (KBr) 3430, 3200, 1730, and 1700 cm^{-1}. The mother liquor from the above crystallisation gave an acidic gum.

Methyl Ester of Isoswazinecic Acid (9).—The acid (9) in tetrahydrofuran was methylated with ethereal diazomethane. Sublimation of the product (50° at 0·03 mmHg) gave the *methyl ester*, m.p. 145° (softens above 130°) (Found: C, 54·5; H, 6·8. $C_{11}H_{16}O_6$ requires C, 54·1; H, 6·6%), $\nu_{max.}$ ($CHCl_3$) 3520, 1790, and 1740 cm^{-1}, *m/e* 226 (8%), 185 (22), 141 (17), 140 (13), 85 (18), 71 (100), and 55 (40), δ (60 MHz; C_5D_5N), 1·30 (3H, d, *J* 7 Hz, $CHCH_3$), 1·57 (3H, s, CCH_3), 2·58 (1H, m, $J_{4.5}$ 5·2 and $J_{5.6}$ 2 Hz, CH_2CHCH), 3·23 (1H, m, $J_{4.7}$ 7 and $J_{4.5}$ 5·2 Hz, CH_3CHCH), 3·70 (3H, s, OMe), 3·90 (2H, d, $J_{5.6}$ 2 Hz, OCH_2CH), and 4·30 (2H, ABq, OCH_2C).

Formation of Isoswazinecic Acid from the Dilactone (1).—The dilactone (1) (1·4 g) and barium hydroxide (12 g) in water (100 ml) were refluxed for 18 h. The mixture was acidified with 2N-sulphuric acid, centrifuged, and continuously extracted with chloroform to give a gummy solid (1·3 g), which, on crystallisation from acetone–chloroform, gave isoswazinecic acid (0·7 g), identified by analysis, m.p., and i.r. spectrum. The mother liquor gave an acidic gum.

Hydrolysis of Swazine by IRA 400 *Resin*.—Swazine (3) (2 g) and IRA 400 resin (OH^- form; 60 ml) in water (100 ml) were kept at 45° for 5 days. The resin was filtered off, washed with water (12 h), and soaked in 2N-sulphuric acid for 24 h. Continuous extraction of the acidic solution with chloroform gave the dilactone (1) (0·44 g), identified by m.p. and i.r. spectrum.

Hydrogenolysis of Swazine (3) *in Ethanol*.—Swazine (3) (2 g) in ethanol (500 ml) was added to Adams catalyst (0·4 g) pre-reduced in ethanol (50 ml) containing 2 drops of concentrated ammonium hydroxide; in 2 h, 485 ml of hydrogen (3·06 mol) were absorbed at 21° and 706 mmHg. Boiling water (200 ml) was added to dissolve precipitated product, and the solution was filtered, and the solvent removed. The product, dried by azeotropic distillation with benzene, was crystallised from absolute ethanol to give 3,3-*epoxymethano*-2-*hydroxy*-2,4-*dimethyl*-5-(7-*methyl*-1-*pyrrolizidinio*-*oxycarbonyl*)*hexanoate* (10) as a fine powder (1·96 g), m.p. 183° (decomp.) (Found: C, 60·4; H, 8·4. $C_{18}H_{29}NO_6$ requires C, 60·8; H, 8·2%), $\nu_{max.}$ (KBr) 3400, 2340, 1730, and 1600 cm^{-1}.

Acid Hydrolysis of the Zwitterion (10).—The zwitterion (10) (1·8 g) in water (50 ml) gave a neutral solution, and this was acidified (pH 1) with 2N-sulphuric acid and continuously extracted with ether for 2 h; evaporation of the ether left a negligible residue. The aqueous solution, further acidified with 3N-sulphuric acid (50 ml), was refluxed for 15 h. Continuous extraction with ether gave a solid (0·8 g), which when crystallised from a small volume of water, gave the isomeric dihydro-spiro-dilactone (see above) as fine needles, m.p. 153—154° (Found: C, 56·3; H, 6·5. $C_{10}H_{14}O_5$ requires C, 56·1; H, 6·6%), $\nu_{max.}$ (KBr) 3400, 1780, and 1755 cm^{-1}, $\nu_{max.}$ ($CHCl_3$) 3570 and 1792 cm^{-1}, *m/e* 214 (M^+, 5%), 126 (63), 111 (20), 98 (50), 72 (25), and 56 (100).

The acidic hydrolysate was basified with barium hydroxide, taken to dryness, and extracted with methanol. Evaporation of the methanol and vacuum sublimation gave retronecanol, m.p. 93—94°, identified by the i.r. spectrum.

Titration of the Dilactones.—The dilactone (1) (209 mg) in water (50 ml) was slowly titrated at room temperature with potassium hydroxide solution (6·20 ml; 0·1575N) using phenolphthalien indicator. The back titration was carried out by refluxing the dilactone (1) (215 mg) in an excess of potassium hydroxide solution (0·1575N; 25 ml) under nitrogen for 12 h and then titrating the excess of base with potassium hydrogen phthalate solution (0·1174N; 17 ml) at room temperature.

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