

86. Steroidal Alkaloids. Part I. The Functional Groups of Cevine.

By D. H. R. BARTON and J. F. EASTHAM.

The steroidal tertiary alkamine, cevine, $C_{27}H_{43}O_8N$, contains a masked secondary α -ketol system, which also appears to be present in the related alkamines germine and protoverine. This system accounts for three of the oxygen atoms; three more are present as non-acylable tertiary hydroxyl groups and one as an easily acylated hydroxyl function; the remaining oxygen is accounted for as a difficultly acylated hydroxyl group.

Cevine and its derivatives have been examined for their susceptibility to glycol cleavage by periodic acid and lead tetra-acetate.

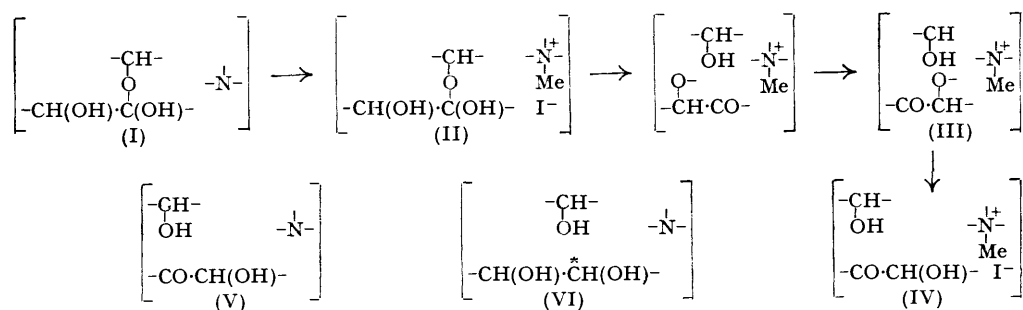
THE steroidal alkamine cevine (see Fieser and Fieser, "Natural Products related to Phenanthrene," 3rd Edn., Reinhold Publ. Corp., p. 600 *et seq.*, and Henry, "The Plant Alkaloids," 4th Edn., J. and A. Churchill Ltd., p. 700 *et seq.*), has the molecular formula $C_{27}H_{43}O_8N$. A study of the far ultra-violet absorption spectrum (cf. Bladon, Henbest, and Wood, *J.*, 1952, 2737) reveals the absence of an ethylenic linkage, whilst the infra-red spectrum (Jaffé and Jacobs, *J. Biol. Chem.*, 1951, **193**, 325; Stoll and Seebeck, *Helv. Chim. Acta*, 1952, **35**, 1270) shows the absence of a carbonyl group. Cevine has all the properties of a tertiary amine; it gives a crystalline oxide with hydrogen peroxide (Freund, *Ber.*, 1904, **37**, 1946) and forms quaternary salts with ease. Further it is a moderately strong base ($pK_b = 4.26$) (Jacobs and Craig, *J. Biol. Chem.*, 1938, **125**, 625; see also the Experimental section). In view of these facts cevine must be heptacyclic and not hexacyclic (cf. Fieser and Fieser, *op. cit.*).

Cevine readily forms diacyl derivatives, thus showing the presence of two primary or secondary hydroxyl groups. On reduction with sodium and butanol or (better) ethanol cevine affords dihydrocevine, whilst an isomeric dihydro-compound is formed on catalytic hydrogenation in the presence of Raney nickel (Jacobs and Craig, *loc. cit.*). Cevine reduces ammoniacal silver nitrate in the cold and Fehling's solution on warming (Freund and Schwarz, *Ber.*, 1899, **32**, 800). It seemed to us, *ab origine*, that all these observations would be best explained if cevine were a masked secondary α -ketol containing the grouping (I). The alternative of a masked aldehyde function was dismissed on the grounds that cevine is stable to hot very concentrated sodium ethoxide (see Experimental). The conclusion was confirmed by the following experiments.

Freund and Schwarz (*J. pr. Chem.*, 1918, **96**, 237) and later Jacobs and Craig (*loc. cit.*) observed that when cevine methiodide was subjected to conditions suitable for Hofmann degradation, the reaction did not pursue the expected path, but that instead a betaine, $C_{28}H_{45}O_8N$, was formed. This betaine gives crystalline salts (Freund and Schwarz, *loc. cit.*) and we have shown specifically that the hydriodide, $C_{28}H_{46}O_8NI$, is not identical with cevine methiodide. We have found that both the betaine and its hydriodide contain carbonyl groups, for they show intense absorption maxima in the infra-red at 1710 cm.^{-1} , values

indicative of a ketone grouping contained in a six-membered ring. These results can be plausibly interpreted by the expressions (II) for cevine methiodide, (III) for the betaine, and (IV) for the derived hydriodide. Thus we formulate the betaine as a secondary α -ketolic betaine; the infra-red spectrum, of course, excludes the possibility (cf. Gäumann and Günthard, *Helv. Chim. Acta*, 1952, **35**, 53) that it could be a carboxylate betaine. The function of the vigorous alkaline conditions in the preparation of the betaine is, therefore, to equilibrate the α -ketol grouping. The two dihydrocevine isomers are to be formulated as stereoisomers at the secondary carbon atom marked with the asterisk in (VI). The one formed under alkaline conditions will have the equatorial hydroxyl, that obtained by hydrogenation the polar hydroxyl group.

Further evidence in support of the masked α -ketol formula for cevine comes from two directions. First, cevine is weakly acidic, forming a crystalline potassium derivative (Hess and Mohr, *Ber.*, 1919, **52**, 1984), advantage of which is taken in the isolation of cevine (cf. Macbeth and Robinson, *J.*, 1922, **121**, 1571). Secondly, the elegant work of Stoll and Seebeck (*loc. cit.*) has led to the important discovery that cevine is *not* the parent alkamine of the ester alkaloids present in the "veratrine alkaloids." The Swiss authors have shown that cevadine and veratridine, previously regarded as ester alkaloids derived from cevine, in fact, give rise on mild alkaline hydrolysis to an isomeric steroidal alkamine cevagenin. The latter is readily isomerised to cevine by strong potassium hydroxide. Cevagenin is a ketone as shown by the infra-red carbonyl frequency at 1707 cm^{-1} identical in position with that of the betaine and its hydriodide (see above). It must be given the partial formula (V). Cevagenin affords a crystalline oxime under neutral conditions whereas cevine does not.



We believe that the steroidal alkaloid germine, $\text{C}_{27}\text{H}_{43}\text{O}_8\text{N}$, which is similar to cevine in many respects (Fieser and Fieser, *op. cit.*), also contains a masked α -ketol system. This is consistent with the isomerisation of germine by alkali to isogermine which, unlike germine, contains a carbonyl group (Fried, Numerof, and Coy, *J. Amer. Chem. Soc.*, 1952, **74**, 3041) and affords an oxime (Jaffé and Jacobs, *loc. cit.*). No doubt the relation between protoverine and isoprotoverine (Jacobs and Craig, *J. Biol. Chem.*, 1943, **149**, 271) is the same as that between germine and isogermine and between cevine and cevagenin.

Although the equilibrium position for the isomeric pair cevine and cevagenin must lie far on the side of cevine, Jaffé and Jacobs (*loc. cit.*) recently reported that cevime (α -cevime) was isomerised to " β -cevime " by alkali. We find that this so-called isomerisation is in fact the autoxidation of a few per cent. of the cevime to give a diosphenol chromophore (see Experimental). In agreement with this view Jaffé and Jacobs found that the infra-red spectra of cevime and " β -cevime " were identical, whilst the rotation of the latter was close to that for pure cevime. We conclude that " β -cevime " is simply slightly impure cevime.

The many oxygen functions in cevime naturally invite enquiry into the susceptibility of the alkamine and its derivatives to attack by glycol-splitting reagents. We have carried out a number of experiments with the results summarised in the Table.

The significance of the extensive consumption of lead tetra-acetate by cevime and dihydrocevime is not at once apparent. Some interesting conclusions were, however, reached from a study of the initial uptake of the reagent. It can be concluded from the

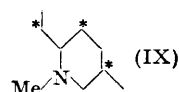
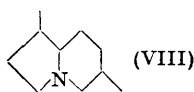
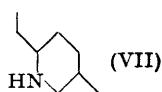
results for cevine, compared with those for dihydrocevine and for the betaine, that one of the two moles of lead tetra-acetate consumed so rapidly by cevine is involved in splitting the masked α -ketol grouping. Further, one of the acetate groups in cevine diacetate must be attached to the secondary hydroxyl group of the masked α -ketol system.*

Compound	No. of moles of oxidant consumed		
	HIO ₄	Pb(OAc) ₄	
	Final uptake after 24 hours	Initial uptake *	Final uptake †
Cevine	2.00	2.03	ca. 8.5
Dihydrocevine	—	1.09	ca. 8.5
Betaine	2.14	1.35	—
Cevine diacetate	1.06	1.00	2.88
Cevine tetra-acetate	—	0	0

* Within 15 sec.; no further uptake in a period of minutes.

† After 30 hours.

Seebeck and Stoll (*loc. cit.*) have recently shown that acetylation of cevine with perchloric acid as catalyst affords a tetra-acetate. We find that this compound is stable to chromic acid and so cannot contain a secondary hydroxyl group. Seebeck and Stoll also showed that cevagenin readily formed a triacetate. This must mean that that hydroxyl group of cevagenin which becomes the ether linkage of cevine is either primary or secondary in character [as already indicated in (I—VI)]. The stability of cevine tetra-acetate to chromic acid oxidation therefore implies, in addition, that both hydroxyl groups of the masked α -ketol system are acetylated. Thus the tetra-acetate contains nine oxygen atoms bound up in acetylated functions; the remaining three must be present as tertiary hydroxyl groups. Cevine diacetate is not stable to chromic acid. This we regard as due to the presence of the unacetylated tertiary hydroxyl group of the masked α -ketol system and/or to the presence of a not readily acetylated secondary hydroxyl group.



As to the relation of the oxygenated functions of cevine to the tertiary nitrogen atom, the following conclusion seems justified. Whilst soda-lime distillation of cevine afforded 2-ethyl-5-methylpiperidine (VII) (Jacobs and Craig, *J. Biol. Chem.*, 1938, **124**, 659) and a dicyclic base, C₁₀H₁₉N, possibly (VIII), similar degradation of the cevine betaine (*idem, ibid.*, 1938, **125**, 625) furnished only a *N*-methyl hydroxy-base, C₉H₁₉ON. The further degradation studies of Jacobs and Craig (*loc. cit.*) on this base indicate, when correctly interpreted, that the hydroxyl group is attached to a carbon atom β with respect to the nitrogen. Thus on Hofmann degradation of the corresponding methohydroxide a basic *oxide*, not an olefin, resulted. At least one of the positions marked with an asterisk in the formula (IX) for the base C₉H₁₉ON must bear a hydroxyl group. Further studies on the constitution of cevine will be reported in due course.†

EXPERIMENTAL

M. p.s are uncorrected. Since the m. p.s of cevine and nearly all its derivatives depend on the rate of heating the standard technique of inserting the m. p. capillary tube 10° below the observed m. p. was adopted. Unless specified to the contrary, rotations were determined in acetone solution at room temperature, which varied from 15° to 25°. Values of $[\alpha]_D$ have been approximated to the nearest degree.

Ultra-violet absorption spectra were measured in ethanol solution with a Unicam S.P. 500 Spectrophotometer. We are indebted to Dr. Keith Freeman of the University of California

* This secondary hydroxyl must be equatorial in character (see Barton, *Experientia*, 1950, **6**, 316) from the method of isolation of cevine (equilibration); it must, therefore, be easily acetylated. In principle the tertiary hydroxyl group of the masked α -ketol system might also be acetylated but, as a working hypothesis, we have tentatively assumed that the second acetate residue in the diacetate is attached elsewhere in the molecule.

† It is our understanding that this problem is also under investigation in the laboratories of the E.T.H., Zurich. We thank cordially Professor V. Prelog and Dr. O. Jeger for this information.

and Dr. A. R. H. Cole of the University of Western Australia for the determination of the infra-red spectra; the measurements were made on a Nujol mull unless specified to the contrary.

Electrometric titrations were carried out in 10% ethanol-water with the Cambridge pH meter.

For periodic acid titrations the procedure advocated by Jackson ("Organic Reactions," Vol. II, p. 361) was adopted. Freshly prepared aqueous solutions of periodic acid were used for each experiment, the alkamine derivative being dissolved in methanol. The composition of the reaction medium was adjusted to 50% aqueous methanol.

For long-term lead tetra-acetate reactions a standard solution, 0.058M with respect to lead tetra-acetate, in "AnalaR" acetic acid was used. The alkamine derivative was dissolved directly in the stock solution. The solutions were titrated according to the procedure of Hockett and McClenahan (*J. Amer. Chem. Soc.*, 1939, **61**, 1670).

For determination of the initial rate of uptake of lead tetra-acetate a somewhat different procedure was adopted. The alkamine derivative (approx. 0.03 mole) was dissolved in 1:1 (by vol.) aqueous acetic acid (1 ml.). To this solution was added with vigorous agitation, during 10 seconds, acetic acid (1 ml.) containing lead tetra-acetate (0.06 mole). After a further *t* seconds 3% potassium iodide in saturated aqueous sodium acetate (5 ml.) was added and the iodine liberated was titrated in the usual way. The appropriate blank determination, which, in fact, showed no consumption of lead tetra-acetate, was carried out in the same way. The time of reaction was regarded as (*t* + 5) seconds. The experiments were also repeated with cevine and dihydrocevine in acetic acid alone without significant change in the uptake of lead tetra-acetate.

Isolation of Cevine.—Cevine was isolated from crude veratrine alkaloids (T. and H. Smith Ltd.) essentially according to Macbeth and Robinson's procedure (*J.*, 1922, **121**, 1571). To precipitate cevine from an aqueous solution of its potassium salt one must reduce the pH to only 9–10 by the addition of (solid) carbon dioxide. Further addition of carbon dioxide redissolves the cevine since it is a sufficiently strong base to be soluble in water at pH 8. Cevine was recrystallised slowly from dilute aqueous alcohol (yield: 35 g. from 100 g. of crude alkaloid esters). It had m. p. 172–176° decomp. (inserted at 160°), $[\alpha]_D -29^\circ$ (*c*, 14.40), -29° (*c*, 5.00), -29° (*c*, 2.60). Macbeth and Robinson (*loc. cit.*) give $[\alpha]_D -30.8^\circ$. The equivalent weight (Found: 573) was determined by electrometric titration with 0.0501N-hydrochloric acid [Calc. for $C_{27}H_{43}O_8N, 3.5H_2O$ (see Freund and Schwarz, *Ber.*, 1899, **32**, 800): 571]. The pH at half-neutralisation was 9.74; pK_b 4.26. In the lead tetra-acetate oxidation experiments anhydrous and hydrated cevine gave the same rate of uptake of the reagent and the same final uptake.

Stability of Cevine to Bases.—Hydrated cevine (1.0 g.) in ethanol (5 ml.; previously saturated with sodium under reflux) was refluxed for 1 hour. Dilution of the solution with water and suitable extraction led to recovery of cevine (0.71 g., 79%), m. p. and mixed m. p. 170–175°, $[\alpha]_D -29^\circ$ (*c*, 3.92).

Attempted Formation of Carbonyl Derivatives from Cevine.—Cevine was recovered unchanged [m. p. 170–178°, $[\alpha]_D -29^\circ$ (*c*, 2.92)] after treatment with ethanolic semicarbazide acetate at room temperature for 5 days or after 2 hours' refluxing. Similar results were obtained with ethanolic 2:4-dinitrophenylhydrazine hydrochloride.

Preparation of Dihydrocevine.—Dihydrocevine, m. p. 260–265° (decomp.) (from concentrated methanolic solution), $[\alpha]_D -26^\circ$ (*c*, 3.3 in MeOH), was prepared as described by Jacobs and Craig (*J. Biol. Chem.*, 1938, **125**, 625) except that ethanol was used instead of butanol. Dihydrocevine was conveniently purified through the *hydrochloride*. Recrystallised from ethanol-chloroform this had m. p. 255° (efferv.), $[\alpha]_D -18^\circ$ (*c*, 1.8 in MeOH), -16° (*c*, 1.4 in MeOH) (Found: C, 59.05; H, 8.2; N, 2.7. $C_{27}H_{46}O_8NCl$ requires C, 59.15; H, 8.45; N, 2.6%). Dihydrocevine, crystallised from methanol, was titrated electrometrically with 0.0584N-hydrochloric acid (Found: equiv., 546. Calc. for $C_{27}H_{45}O_8N, CH_3 \cdot OH$: equiv., 539). The pH at half-neutralisation was 9.65; pK_b 4.35. Dihydrocevine showed no selective absorption in the ultra-violet (even in the far ultra-violet) region, and showed no carbonyl band in the infra-red. Dihydrocevine does *not* reduce ammoniacal silver nitrate or Fehling's solution.

The Betaine from Cevine Methiodide.—Cevine methiodide, m. p. 250–253°, $[\alpha]_D -5^\circ$ (*c*, 1.70 in MeOH), was prepared according to Jacobs and Craig's method (*loc. cit.*; cf. Freund and Schwarz, *J. pr. Chem.*, 1917, **96**, 236). It showed no carbonyl absorption in either the ultra-violet or the infra-red region. The derived betaine was prepared essentially according to Jacobs and Craig's method (*loc. cit.*) except that it was found necessary to heat the alkaline solution at 115° (internal temp.) in an oil-bath rather than on the steam-bath. The betaine,

after digestion with aqueous ethanol, had m. p. 265—268°, λ_{\max} . 280 μ (ϵ , 70), and showed an intense carbonyl band at 1710 cm^{-1} in the infra-red.

The betaine was transformed into its hydrochloride, m. p. 245°, $[\alpha]_D -29^\circ$ (c , 2.00 in MeOH), λ_{\max} . 280 μ (ϵ , 58), as described by Jacobs and Craig (*loc. cit.*), and into its hydriodide by the following procedure. The betaine (300 mg.) in acetic acid (0.5 ml.) was admixed with aqueous potassium iodide (20%; 1 ml.). Crystallisation was induced by scratching. Next morning the highly crystalline *hydriodide* (280 mg.) had m. p. 250° (decomp.), $[\alpha]_D -27^\circ$ (c , 1.91 in MeOH), λ_{\max} . 280 μ (ϵ , 60) (Found: C, 51.25; H, 7.15; N, 2.2; I, 19.9. $\text{C}_{28}\text{H}_{46}\text{O}_8\text{NI}$ requires C, 51.6; H, 7.1; N, 2.2; I, 19.5%). The betaine hydriodide was mentioned by Freund and Schwarz (*J. pr. Chem.*, 1917, **96**, 236) who found m. p. 256° (decomp.) but gave no analytical data. On electrometric titration the betaine hydriodide behaved as a quaternary ammonium halide. Both the betaine hydrochloride and hydriodide showed intense carbonyl maxima in the infra-red region at 1710 cm^{-1} .

Cevine Diacetate.—The diacetate, prepared as described by Freund (*Ber.*, 1904, **37**, 1946) and conveniently crystallised from ether, had m. p. 188—191°, $[\alpha]_D -4.5^\circ$ (c , 15.5). It showed no ketonic or double-bond ultra-violet absorption (Found: C, 62.55; H, 8.4; N, 2.25; Ac, 14.85. Calc. for $\text{C}_{31}\text{H}_{47}\text{O}_{10}\text{N}$: C, 62.75; H, 8.0; N, 2.35; Ac, 14.5%).

Cevine diacetate (211 mg., 0.36 mmole) was treated with chromium trioxide (172 mg.) in water (10 ml.) and "AnalaR" acetic acid at 5°. With the aid of the appropriate blank the rate of consumption of chromic acid was shown to be as follows: 5 hr., 0.30 m.equiv.; 24 hr., 0.46 m.equiv.; 72 hr., 0.68 m.equiv. (theor. for 1 O: 0.72 m.equiv.).

Cevine Tetra-acetate.—Cevine tetra-acetate, m. p. 290—295°, $[\alpha]_D +38^\circ$ (c , 3.56 in EtOH), and its perchlorate were prepared by Stoll and Seebeck's method (*loc. cit.*). The physical constants found by us are in good agreement with those recorded by them except that the perchlorate was found to have a double m. p. at 240° and 268°. Material melting at 240° is obtained when the salt is crystallised from water, but even this, if inserted below 240° and heated slowly, will fail to melt until about 260°. Material melting at 268° (decomp.) is obtained when the salt is crystallised from glacial acetic acid. A mixture of the two forms melts at the higher temperature without depression. The tetra-acetate shows no double-bond absorption in the far ultra-violet region. It was reconverted into cevine by alkaline hydrolysis.

Cevine tetra-acetate perchlorate (132 mg.) was treated with chromium trioxide (62 mg.) in water (3 ml.) and "AnalaR" acetic acid (5 ml.) at 5°. During 24 hours the consumption of chromic acid was <10% of that required for one atom of oxygen. Cevine tetra-acetate was recovered (70%) unchanged from the solution and characterised as the perchlorate, m. p. 240°.

Autoxidation of Cevine in Alkaline Solution.—A solution of "AnalaR" sodium hydroxide (1.5 g.) in water (15 ml.; oxygen-free) and ethanol (100 ml.; oxygen-free) was prepared. Cevine (200 mg.) was dissolved in this solution (20 ml.). In two parallel experiments one batch of cevine solution was kept under nitrogen in a stoppered flask, the other briefly aerated with carbon dioxide-free air and then stored under carbon-dioxide-free air (*cf.* Jaffé and Jacobs, *loc. cit.*). After 12 days the two solutions, after dilution with the appropriate amount of 0.05N-sodium hydroxide in 95% ethanol, had λ_{\max} . 300—305 μ (ϵ , 70 and 710 respectively). The latter intensity is far too great to be due to an isolated carbonyl group and the position of the maximum is also somewhat displaced. When the solutions were acidified with concentrated hydrochloric acid the position of the maxima at once changed to 275—280 μ and the intensity of absorption increased by 15% in both cases. The shift, and, in particular, the change in relative intensity are characteristic of the diosphenol chromophore rather than of the phenol chromophore (Eastham, to be published) where the relative intensity shift is inverted. Diosphenols have λ_{\max} . 10,000 \pm 3000 so that only a few per cent. of diosphenol are produced in the autoxidation. In several repetitions of these experiments on a larger scale cevine was recovered unchanged in good yield. In a typical experiment it had m. p. 163—168°, $[\alpha]_D -30^\circ$ (c , 1.64), and showed no selective ultra-violet absorption.

We thank the Government Grants Committee of the Royal Society, the Central Research Fund of London University, the Chemical Society, and Imperial Chemical Industries Limited for financial assistance. This work was carried out during the tenure of an Atomic Energy Commission (America) Post-doctorate Research Fellowship by one of us (J. F. E.).