Steroidal Alkaloids. Part III. The Constitution and Stereochemistry of Cevine.*

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Methanolysis of cevine orthoacetate triacetate affords cevine orthoacetate diacetate, oxidised by chromic acid to a ketone. A similar oxidation is effected on cevadine orthoacetate acetate. These experiments show that cevine contains no primary hydroxyl group.

The constitutions of various acetylated cevadine derivatives are discussed and the possible presence of an enol acetate grouping is considered.

Catalytic hydrogenation of cevine orthoacetate triacetate affords cevine "dihydro-orthoacetate" triacetate in which the presence of an ethylenedioxy-group has been detected. This confirms the presence of an orthoacetate grouping in the precursor.

Reduction of cevine orthoacetate triacetate with lithium and liquid ammonia furnishes a dihydrocevine orthoacetate, the triacetate of which is stable to chromic acid. This and other evidence confirms that the terminus of the oxide ring in cevine is derived from a tertiary hydroxyl group.

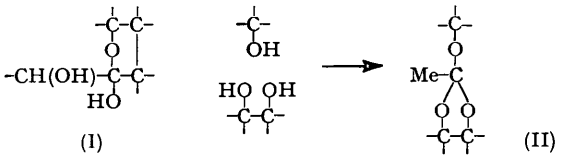
Evidence is presented that the orthoacetate system of cevadine orthoacetate diacetate and related compounds is attached both α and β with respect to the 16-hydroxyl group.

Treatment of dihydrocevine orthoacetate with dilute sulphuric acid affords an isomeric orthoacetate. The bearing of this and other observations on the stereochemistry of cevine is briefly discussed.

In Parts I and II of this series * the oxygenated functional groups of the steroidal alkamine cevine, $C_{27}H_{43}O_8N$, were shown to be as follows : three oxygen atoms bound up in a masked

* Part I and II, *J.*, 1953, 424; 1954, 2137.

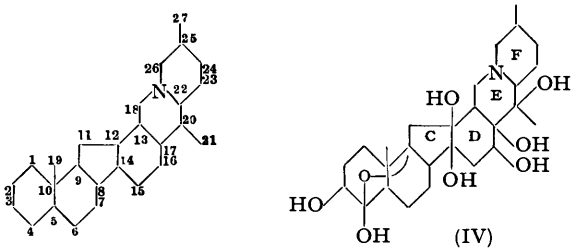
secondary α-ketol system (I); two more present as a ditertiary α-glycol system related in space to a third tertiary hydroxyl in such a way as to explain ready formation of an ortho-acetate (II); the remaining two oxygen atoms present one as a primary or secondary hydroxyl group and one as a tertiary hydroxyl group. We commence the present paper by clarifying the final obscurity in the nature of the functional groups.



(I) (II)

Methanolysis of cevine orthoacetate triacetate, which is known to be base-catalysed (see Rosenfelder, *J.*, 1954, 2638), removed one acetate residue preferentially, affording cevine orthoacetate diacetate. The ease of this reaction implies that the acetate residue removed was attached to a primary or a secondary hydroxyl group. That the latter is correct was demonstrated by titrimetric oxidation of cevine orthoacetate diacetate to a ketone. A primary hydroxyl group cannot, therefore, be present in cevine.

Whilst the work on the functional groups summarised in this paper and in Parts I and II was in hand parallel studies on the more vigorous degradation of the cevine molecule were in progress in other laboratories. On the basis of dehydrogenation evidence Jacobs and Pelletier proposed (*J. Org. Chem.*, 1953, **18**, 765) and supported (*J. Amer. Chem. Soc.*, 1954, **76**, 2028) the fundamental skeleton (III) for cevine and related alkaloids. In the laboratories at Zürich and at Harvard elegant oxidative degradational studies of the cevine molecule have been pursued, of the progress of which we have, from time to time, been informed. Summing the evidence available, both experimental and interpretative, made structure (IV) very plausible for cevine. In collaboration with Dr. O. Jeger and Professor



(III) (IV)

V. Prelog of Zürich and with Professor R. B. Woodward of Harvard, the arguments in favour of this structure have recently been summarised [Barton, Jeger, Prelog, Woodward (and their respective collaborators), *Experientia*, 1954, **10**, 81]. We express here our cordial appreciation of the friendly international exchange of facts and theories which eventually made possible this joint paper. It is not our intention to repeat now any of the closely woven skein of logic used in the derivation of structure (IV) and we content ourselves with summarising the relevant, hitherto unpublished experiments, carried out at Birkbeck College, which may be adduced as support for the formula.

Treatment of cevadine (V; R = angeloyloxy) with acetic anhydride–perchloric acid affords cevadine orthoacetate diacetate (Stoll and Seebeck, *Helv. Chim. Acta*, 1952, **35**, 1942; Kupchan, Lavie, Deliwala, and Andoh, *J. Amer. Chem. Soc.*, 1953, **75**, 5519). This can be selectively methanolysed to cevadine orthoacetate acetate and we find that titrimetric chromic acid oxidation of the monoacetate affords a ketone stable to further attempted oxidation. This confirms the absence of a primary hydroxyl group in cevine (cf. above). Careful alkaline hydrolysis (Stoll and Seebeck, *loc. cit.*) of cevadine orthoacetate acetate affords cevadine orthoacetate.

The infra-red spectra (all in CS_2) of this series of compounds (see Table 1) revealed that their structural basis might be more complicated than indicated by the names given above.

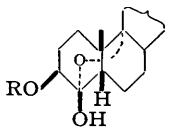
Thus all the compounds retaining the acetate grouping at $C_{(4)}$ showed marked shifts of the bands for the 4-acetate and the 4-angelate group relative to the expected positions. It has been suggested to us by Dr. O. Jeger (personal communication) that the simplest interpretation of these shifts is that the 4-acetates are really enol-acetates. Thus cevadine orthoacetate diacetate is possibly to be formulated as cevadine orthoacetate enol-acetate acetate

TABLE 1. *Infra-red maxima* (cm.$^{-1}$).

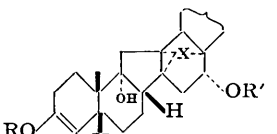
Compound	Angelate C:O	Angelate C:C	(?) Enol angelate C:O	(?) Enol angelate C:C	Acetate C:O	(?) Enol acetate C:O
Cevadine	1705 1232	1640	—	—	—	—
Cevadine orthoacetate diacetate	—	—	1722 1230	1640	1738 1244	1764 1216
Cevadine orthoacetate acetate	—	—	1725 1232	1644	—	1765 1216
Dihydrocevadine orthoacetate acetate	—	—	—	—	—	1762 1222
Cevadine orthoacetate	1708 1230	1638	—	—	—	—
Ketone (XII; R = angeloyloxy) from cevadine orthoacetate acetate	—	—	1723 1238	1640	—	1760 1218

(VI; R = angeloyloxy, R′ = Ac), and cevadine orthoacetate acetate as cevadine orthoacetate enol-acetate (VI; R = angeloyloxy, R′ = H). In cevadine orthoacetate the carbonyl frequency is again normal, which would indicate re-closure to the original masked α-ketol system as in (VII; R = angeloyloxy).

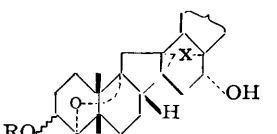
In an endeavour to confirm the postulated enol-acetate structures, cevadine orthoacetate was acetylated by pyridine–acetic anhydride to give a new compound which we regard as acetylated at $C_{(16)}$. Further acetylation with acetic anhydride–perchloric acid afforded the known cevadine orthoacetate diacetate, and not a ring-A closed isomer. Also treatment of cevadine orthoacetate acetate with N-sulphuric acid did not provoke isomerisation to a new orthoacetate system as might have been expected (see below) if a free 9-hydroxyl group had been available.



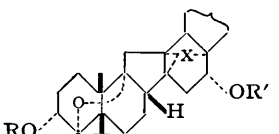
(V)



(VI)

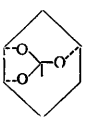


(VII)



(VIII)

The ring containing X is to be understood as being the grouping (*A*).

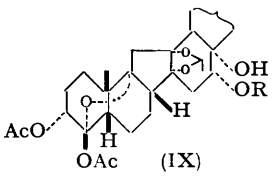


(*A*)

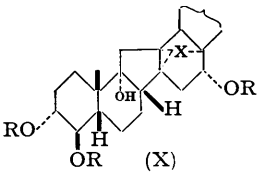
Catalytic hydrogenation of the perchlorate of cevine orthoacetate triacetate (VIII; R = R′ = R″ = Ac) in acetic acid with a platinum catalyst furnished a cevine " dihydro-orthoacetate " triacetate which we formulate as the ethylenedioxy-compound (IX; R = Ac) on the basis of the following evidence. Acetyl determination showed the presence of only three acetic acid residues; acid hydrolysis afforded acetaldehyde, isolated as the 2 : 4-dinitrophenylhydrazone; oxidation with chromic acid gave back cevine orthoacetate triacetate. Partial methanolysis furnished cevine " dihydro-orthoacetate " diacetate (IX; R = H). These experiments may be taken as confirmatory for the formulation of " an-

hydrocevine " as cevine orthoacetate (Part II, *loc. cit.*; see also Stoll and Seebeck, *Helv. Chim. Acta*, 1954, **37**, 824).

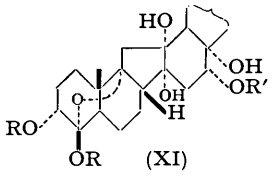
One of the features of cevine chemistry about which it was difficult to furnish a direct proof in Parts I and II was the terminus of the oxide ring (now secured at $C_{(9)}$, see above). We originally concluded (see Part II) that this affords a tertiary hydroxyl group since cevagenin orthoacetate diacetate is stable to chromic acid. Although the interpretation of this experiment is subject to qualification (see below) the conclusion reached is correct and is confirmed as follows. Reduction of cevine orthoacetate triacetate (VIII; R = R′ = R″ = Ac) with lithium and liquid ammonia gave dihydrocevine orthoacetate (X; R = H), which readily afforded a triacetate (X; R = Ac) on acetylation with pyridine and acetic



(IX)



(X)



(XI)

anhydride. This triacetate was stable to chromic acid and therefore cannot contain an unacetylated (C_7) secondary hydroxyl group. The assigned β-configuration at $C_{(4)}$ in dihydrocevine orthoacetate triacetate (X; R = Ac) is based on the presumed carbanionic mechanism of reduction (see Barton and Robinson, *J.*, 1954, 3045).

A structural feature of some uncertainty in the cevine formula (IV) of Barton, Jeger, Prelog, and Woodward (*loc. cit.*) is the attachment of a tertiary hydroxyl at $C_{(17)}$ rather than at $C_{(13)}$. We are now able to provide firm evidence as to the correctness of the formulation of ring D in (IV). Selective methanolysis of cevine 3 : 4 : 16-triacetate (XI; R = R′ = Ac) gave cevine 3 : 4-diacetate (XI; R = Ac, R′ = H). Chromic acid oxidation then furnished a ketone which showed an infra-red maximum at 1702 $cm.^{-1}$ (6-ring ketone) in agreement with the assigned constitution. When, however, cevine orthoacetate diacetate (VIII; R = R″ = Ac, R′ = H) (see above) was similarly oxidised the derived ketone was shown by quantitative infra-red spectroscopy to have the carbonyl band displaced to 1730 $cm.^{-1}$. Such a displacement could only be explained by a change in α- [as in (VIII)], not β-, substitution.

In agreement with these structures stand experiments with lead tetra-acetate and with periodic acid summarised in Tables 2 and 3. In order to conserve space we do not present

TABLE 2. *Lead tetra-acetate oxidations.*

Compound oxidised	Mols. of oxidant added	Mols. of oxidant consumed at various times
Cevadine	3·0	1·0 (10 min.); 1·5 (5 hr.); 3·0 (16 hr.)
Cevadine orthoacetate	3·0	0·0 (2 hr.); 0·0 (16 hr.)
Cevadine orthoacetate acetate	3·0	0·0 (2 hr.); 0·0 (16 hr.)
Ketone (XII; R = angeloyloxy)	3·4	0·0 (2 hr.); 0·0 (16 hr.)
Cevine triacetate (see Part II)	4·0	0·95 (10 min.); 0·95 (18 hr.)
Cevine 3 : 4-diacetate	3·0	0·95 (10 min.); 1·8 (5 hr.); 3·0 (16 hr.)
Cevine 3 : 16-diacetate	3·3	0·97 (10 min.); 2·5 (4 days)
Cevine 16-acetate perchlorate (see Exptl.)	3·2	2·0 (10 min.); 2·0 (1 hr.); 2·9 (4 days)
Ketone from cevine 3 : 4-diacetate	3·4	1·5 (10 min.); 2·4 (3 hr.); 3·4 (20 hr.)

a detailed discussion of the results, but trust that the interpretation will be clear from the formulæ given. We would however direct attention to one especially striking set of results. Cevine 3 : 4 : 16-triacetate (XI; R = R′ = Ac) consumes only one mol. of lead tetra-acetate even on long treatment, but the derived 3 : 4-diacetate (XI; R = Ac, R′ = H) takes up nearly two mols. in 5 hours in agreement with the view that partial methanolysis of the triacetate reveals a cleavable glycol system (at position 16 : 17). The ketone from oxidation of cevine 3 : 4-diacetate behaved similarly.

A proof that one end of the orthoacetate system in cevine derivatives was placed β with

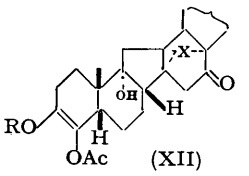
respect to the 16-hydroxyl group was obtained as follows The ketone (XII; R = angeloyloxy) obtained (see above) by oxidation of cevadine orthoacetate acetate (VI; R = angeloyloxy, R′ = H) was sensitive to base. On treatment with aqueous-methanolic sodium hydroxide at room temperature for a short period the ketone afforded an αβ-unsaturated ketone (XIII; R = angeloyloxy). The loss of the orthoacetate residue can only be explained by β-elimination (at $C_{(14)}$) as provided for by the above-cited formulæ.

TABLE 3. *Periodic acid oxidations.*

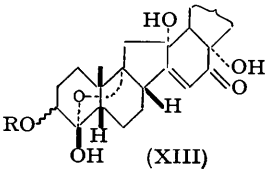
Compound oxidised	Mols. of oxidant added	Mols. of oxidant consumed at various times	
		No $NaHCO_3$.*	$NaHCO_3$ added.
Cevadine	2·0	0·90 (2—25 min.)	—
Cevine	4·5	2·0 (10 min.); 2·0 (24 hr.)	2·7 (15 min.); 3·5 (1 hr.); 4·5 (2 hr.)
Cevagenin	4·5	2·0 (1 hr.); 2·0 (2 hr.)	1·5 (15 min.); 2·5 (2 hr.); 4·5 (6 hr.)
Cevine triacetate	5·0	1·0 (10 min.); 1·0 (16 hr.)	0·9 (10 min.); 3·2 (16 hr.)
Cevine tetra-acetate	4·5	—	0·0 (16 hr.)
Dihydrocevine orthoacetate	4	0·9 (10 min.); 0·95 (45 min.)	1·03 (45 min.)
Unsaturated ketone (XIII; R = angeloyloxy)	4·5	0·0 (2—60 min.)	0·16 (10 min.); 0·7 (70 min.); 2·0 (4 hr.)

* As shown on p. 3958 periodic acid oxidations of cevine derivatives are very dependent on pH. With periodic acid itself little, if any, oxidation occurs until sodium hydrogen carbonate is added just before titration. These experiments therefore measure the "instantaneous" periodic acid consumption.

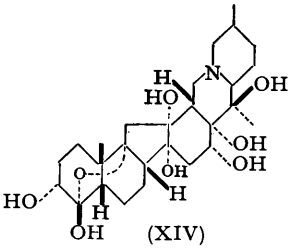
Catalytic hydrogenation gave the dihydro-derivative (XIII; R = α-methyl-*n*-butyryl) showing $\lambda_{max.}$ 238 mμ (ε 12,500) in agreement with the assigned structure. The latter compound was also obtained by subjecting the ketone from dihydrocevadine orthoacetate acetate (XII; R = α-methyl-*n*-butyryl) to mild treatment with alkali. In the infra-red, the ketone (XIII; R = angeloyloxy) showed bands (in $CHBr_3$) at 1700 and 1240 (αβ-unsaturated ester grouping) and at 1672 $cm.^{-1}$ (αβ-unsaturated ketone), whilst its dihydro-derivative showed bands (in $CHBr_3$) at 1720 and 1260 (ester grouping) and at 1670 $cm.^{-1}$ (αβ-unsaturated ketone), both bands being of such an intensity as to correspond with only one carbonyl group. The infra-red data confirm that no extra ketonic grouping is produced in the genesis of these αβ-unsaturated ketones.



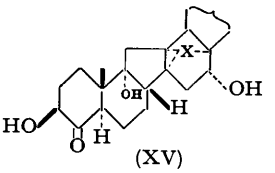
(XII)



(XIII)



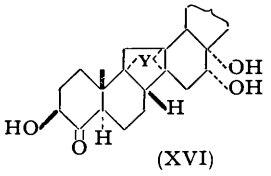
(XIV)



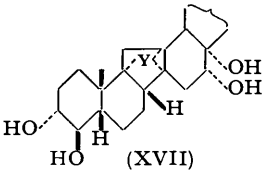
(XV)

The stereochemistry of cevine has already been discussed in outline by Barton, Jeger, Prelog, and Woodward (*loc. cit.*). The configurations at positions 3, 4, 5, 9, and 10 as in (XIV) may be taken as established and one may presume that the configuration at $C_{(8)}$ is β as in all other naturally occurring steroids. The α-configurations at positions 12, 14, and 17

are demonstrated by the following evidence. Stoll and Seebeck (*Helv. Chim. Acta*, 1952, **35**, 1942) reported that alkaline treatment of cevadine orthoacetate acetate afforded a cevagenin orthoacetate (amorphous, $[\alpha]_D$ +34° in EtOH), which would now be formulated (see Barton, Jeger, Prelog, and Woodward, *loc. cit.*) as (XV). Stoll and Seebeck later (*Helv. Chim. Acta.*, 1954, **37**, 824) stated that a repetition of essentially the same reaction conditions gave a crystalline cevagenin orthoacetate (m. p. 184—190°, $[\alpha]_D$ — 39° in EtOH). Both preparations of " cevagenin orthoacetate " gave crystalline cevagenin orthoacetate diacetate on acetylation. It was suggested to us by Dr. S. M. Kupchan (personal communication, *J. Amer. Chem. Soc.*, in the press) that the orthoacetate system of cevagenin orthoacetate, which is produced under mild acetylation conditions, might be in a different position from that which it occupies in cevine orthoacetate, which is obtained under strongly acid-catalysed conditions. According to Kupchan (*loc. cit.*) cevagenin orthoacetate is now to be formulated as (XVI). Treatment of dihydrocevine orthoacetate (X; R = H) with N-sulphuric acid or more simply, but more slowly, its dissolution in acetic acid, afforded a dihydrocevine *iso*orthoacetate (XVII) showing no carbonyl band in the infra-red but giving

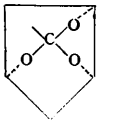


(XVI)



(XVII)

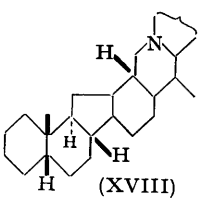
The ring containing Y is to be understood as being the grouping (*B*).



(*B*)

one mol. of acetic acid on acetyl determination. A repetition of the two groups of experiments reported by Stoll and Seebeck (*loc. cit.*) then revealed that it was extraction into N-sulphuric acid that permitted the rearrangement of the initially produced (amorphous and dextrorotatory) cevagenin 12 : 14 : 17-orthoacetate into the more stable 9 : 12 : 14-orthoacetate. The existence of these two series of orthoacetates proves that the 12-, 14-, and 17-hydroyl groups must be on the same (α-) side of the molecule as the 9-hydroxyl group. The same conclusion has also been drawn by Kupchan from his work referred to above. Also since hydroxyl groups at positions 7, 12, and 14 could not furnish an orthoacetate system (see models), the ether bridge of cevine could not terminate at the (secondary) 7-position, a conclusion already reached on other grounds (Barton, Jeger, Prelog, and Woodward, *loc. cit.*; see also above).

Cevine triacetate (see Table 3) is, after the consumption of one mol. of reagent, stable indefinitely to the action of lead tetra-acetate. It seems to us that this is only reasonable if the exposed ditertiary α-glycol system at C_{17} and $C_{(20)}$ is diaxial (cf. Criegee *et al.*, *Annalen*, 1933, **507**, 159; *Ber.*, 1940, **73**, 563), as already indicated. A diaxial arrangement for the 17 : 20-glycol system also fixes the configuration at $C_{(13)}$ (see models) in such a way as to give a *trans*-fusion of rings D and E.



(XVIII)

Finally we discuss the configuration at $C_{(16)}$ which was tentatively regarded as α by Barton, Jeger, Prelog, and Woodward (*loc. cit.*) on the basis of the ready methanolysis of compounds acetylated at this position. The faster cleavage of the 16 : 17-glycol system than of the 17 : 20-system (see Table 2) supports this view, but we are not able to offer conclusive evidence on this point of stereochemistry

In conclusion we advance nomenclature proposals which will probably be applicable also for zygadenine, germine, and protoverine. We suggest that the saturated compound represented by (XVIII) [for numbering see (III) above] should be called cevan. Cevine itself then becomes 4α : 9α-epoxycevan-3α : 4β : 12α : 14α : 16α : 17α : 20β-heptaol.

Experimental

For general experimental details see Part I (*J.*, 1953, 424). Unless specified to the contrary, rotations were determined in chloroform solution at room temperature, and light-absorption

maxima in ethanol (Unicam S.P.500 Spectrophotometer). Infra-red spectra were kindly determined by Messrs. Glaxo Laboratories Ltd. in carbon disulphide solution except as otherwise stated. Acetyl and other volatile-acid determinations were carried out according to Pregl's " Quantitative Organic Microanalysis " (by H. Roth; J. and A. Churchill Ltd., London, 1937).

Methanolysis of Cevine Orthoacetate Triacetate.—Cevine orthoacetate triacetate (2·0 g.) in methanol (100 ml.) was left at room temperature for 7 hr., after which water (20 ml.) was added and the solution left overnight. The solvent was removed under reduced pressure and the residue extracted with chloroform. Crystallisation from aqueous methanol gave *cevine orthoacetate diacetate* (600 mg.), m. p. 283—285°, $[\alpha]_D$ +104° (*c*, 2·14) (Found: C, 63·45; H, 7·7; Ac, 19·7, 19·9. $C_{33}H_{47}O_{10}N,\frac{1}{2}CH_3{\cdot}OH$ requires C, 63·5; H, 7·8; Ac, 20·35%).

Cevine orthoacetate diacetate (400 mg.; dried *in vacuo*) in " AnalaR " acetic acid (45 ml.) containing chromium trioxide (100 mg.) was left at room temperature. After 3 hr. two equivalents of oxidant had been consumed (unchanged on further standing). After working up as for the corresponding oxidation of cevadine orthoacetate acetate (see below), the product was crystallised from chloroform–methanol, to give the required *ketone* (200 mg.) as long needles, m. p. 275—276° (decomp.), $[\alpha]_D$ +98° (*c*, 1·20) (Found: C, 64·0; H, 7·3. $C_{33}H_{45}O_{10}N$ requires C, 64·35; H, 7·35%).

Chromic Acid Oxidation of Cevadine Orthoacetate Acetate.—Cevadine orthoacetate acetate, prepared according to Stoll and Seebeck's method (*Helv. Chim. Acta*, 1952, **35**, 1942), had m. p. 283—285° (decomp.), $[\alpha]_D$ +79° (*c*, 1·20 in EtOH), +72° (*c*, 1·33 in $CHCl_3$), +74° (*c*, 1·98 in $CHCl_3$) [Found: equiv. (determined by volatile acid produced on acid hydrolysis), 224. Calc. for $C_{36}H_{51}O_{10}N$ (one angelate and two acetate residues): equiv., 219].

(*a*) Cevadine orthoacetate acetate (200 mg.) was treated at room temperature with a solution of chromium trioxide (25 ml., 0·065N; five times the amount required for the oxidation of one secondary hydroxyl group) in " AnalaR " acetic acid containing 0·2% of water. Aliquot parts were titrated at intervals. After 2 hr. 95% of the theoretical amount of reagent had been consumed; there was no further uptake after another hour. The residual solution (21 ml.) was treated with a slight excess of aqueous sodium hydrogen sulphite at 10° and then with a slight excess of aqueous ammonia. Extraction with chloroform (6 × 30 ml.) and crystallisation from ethanol afforded the *ketone* (106 mg.) (XII; R = angeloyloxy) as fine needles m. p. 269—270° (decomp.), $[\alpha]_D$ +60° (*c*, 0·98), +58° (*c*, 1·24), +59° (*c*, 1·10) [Found: C, 65·65; H, 7·4; N, 2·6%; equiv. (by potentiometric titration), 663. $C_{36}H_{49}O_{10}N$ requires C, 65·95; H, 7·55; N, 2·15%; equiv., 656]. Treatment of this ketone (50 mg.) with chromium trioxide (twice the amount calc. for the oxidation of one secondary hydroxyl group) in " AnalaR " acetic acid (10 ml.) at room temperature for 4½ hr. resulted in no significant uptake of chromic acid (titration); the ketone was recovered unchanged (m. p., mixed m. p., and rotation).

(*b*) For larger-scale oxidations the following procedure was advantageous. Cevadine orthoacetate acetate (3·54 g.) in 95 : 5 (v/v) carbon tetrachloride–acetic acid (100 ml.) was treated with chromium trioxide solution (50 ml.; 0·66N) in 98·5% aqueous acetic acid for 1 hr. at room temperature, the reaction being controlled by titrations. After being worked up as outlined above, the crude product crystallised from ethanol to give the pure ketone (2·0 g., 59%).

Acetylation of Cevadine Orthoacetate.—Cevadine orthoacetate (Stoll and Seebeck, *Helv. Chim. Acta*, 1952, **35**, 1942) (230 mg.) in acetic anhydride (4 ml.) and pyridine (2 ml.) was heated on the steam-bath for 2 hr. Crystallisation of the product from ether–light petroleum (b. p. 40—60°) gave *cevadine orthoacetate 16-acetate*, m. p. 165—170°, $[\alpha]_D$ +83° (*c*, 1·09) [Found: C, 65·1, 65·45; H, 7·6, 6·8; N, 2·4%; equiv., 224. $C_{36}H_{51}O_{10}N$ requires C, 65·75; H, 7·8; N, 2·15%; equiv. (based on one orthoacetate, one acetate, and one angeloyloxy-residue), 219].

Cevadine orthoacetate 16-acetate (80 mg.) in acetic anhydride (4 ml.) was cooled to −60° and perchloric acid (70%; 0·4 ml.) added. The solution was left at −5° overnight. Crystallisation of the product from ether gave cevadine orthoacetate diacetate, identified by m. p., mixed m. p. and rotation.

Cevadine Triacetate.—Cevadine orthoacetate diacetate (200 mg.) in aqueous acetic acid (50%; 5 ml.) was left at room temperature for 2 hr., diluted with 500 ml. of water, and left for a further hour. Crystallisation of the product from aqueous acetone afforded *cevadine triacetate*, m. p. 265—266°, $[\alpha]_D$ +16° (*c*, 1·14) (Found: C, 62·8; H, 7·3; N, 1·85. $C_{38}H_{55}O_{12}N,\frac{1}{2}H_2O$ requires C, 62·8; H, 7·75; N, 1·9%). Heating of the triacetate (50 mg.) in " AnalaR " acetic acid (2 ml.) on the steam-bath for 10 min. gave back cevadine orthoacetate diacetate, identified by m. p., mixed, rotation, and crystal form.

Hydrogenation of Cevine Orthoacetate Triacetate Perchlorate.—The perchlorate (200 mg.) in " AnalaR " acetic acid (25 ml.) was hydrogenated overnight with a platinum catalyst. After

filtration most of the acetic acid was removed *in vacuo*, and the product was treated with water and adjusted to pH 9 with ammonia. Extraction with chloroform afforded *cevine " dihydro-orthoacetate " triacetate* as needles from chloroform–light petroleum (b. p. 60—80°), m. p. 270—273° (decomp.), $[\alpha]_D$ +58°, +60° (*c*, 0·88, 1·96 respectively in acetone), +59° (*c*, 1·37 in $CHCl_3$) (Found : C, 63·1; H, 7·8; Ac, 19·7, 20·1. $C_{35}H_{51}O_{11}N$ requires C, 63·5; H, 7·75; Ac, 19·5%). This compound was recovered unchanged on further attempted hydrogenation (with or without the addition of excess of perchloric acid) and was not acetylated by pyridine–acetic anhydride at steam-bath temperature. It was however also obtained by the hydrogenation of cevine ortho-acetate triacetate under the same conditions. As would be expected, cevine triacetate was unaffected on attempted hydrogenation as above. Cevine " dihydro-orthoacetate " triacetate reduced Fehling's solution at the same rate as did its progenitor.

The " dihydro-orthoacetate " (125 mg.) in " AnalaR " acetic acid (40 ml.) containing chromium trioxide (50 mg.) was left at room temperature. Aliquot parts were titrated and after 2 hr. the uptake of chromic acid ceased at the consumption of two equivalents. After 5 hr. the solution was worked up to give, on crystallisation from ether, cevine orthoacetate triacetate, identified by m. p., mixed m. p., rotation {$[\alpha]_D$ +121° (*c*, 1·03)}, analysis (Found : C, 63·45; H, 7·7; N, 2·6. Calc. for $C_{35}H_{49}O_{11}N$: C, 63·7; H, 7·5; N, 2·1%), and infra-red spectrum (identical in every detail with that of authentic material).

Detection of an Ethylidene Grouping in Cevine " Dihydro-orthoacetate " Triacetate.—The " dihydro-orthoacetate " (210 mg.) in methanol (7 ml.) was treated with 2 : 4-dinitrophenyl-hydrazine (70 mg.) in concentrated hydrochloric acid (1 ml.) on the steam-bath for 30 min. Dilution with water, extraction with chloroform, and filtration in 1 : 1 benzene–light petroleum (b. p. 40—60°) through alumina, and elution with benzene gave acetaldehyde 2 : 4-dinitrophenyl-hydrazone, identified by m. p., mixed m. p., absorption spectrum ($\lambda_{max.}$ 357 mμ, ε 21,100 in $CHCl_3$), and analysis (Found : C, 42·95; H, 4·1; N, 24·0. Calc. for $C_8H_8O_4N_4$: C, 42·85; H, 3·6; N, 25·0%).

Methanolysis of Cevine " Dihydro-orthoacetate " Triacetate.—The " dihydro-orthoacetate " (200 mg.) in methanol (20 ml.) was left at room temperature for 7 hr. Water (7 ml.) was added and the solution set aside overnight. The solvent was removed *in vacuo* and the crystalline precipitate was collected. Recrystallisation from aqueous methanol gave prisms of *cevine " dihydro-orthoacetate " diacetate*, m. p. 231—233°, $[\alpha]_D$ +50° (*c*, 1·73) (Found : C, 63·1; H, 7·85; N, 2·6; Ac, 14·05. $C_{33}H_{49}O_{10}N,\frac{1}{2}CH_3{\cdot}OH$ requires C, 63·3; H, 8·1; N, 2·2; Ac, 13·5%).

Stability of Cevagenin Orthoacetate Diacetate to Chromic Acid.—Cevagenin orthoacetate diacetate (57 mg.) (Stoll and Seebeck, *Helv. Chim. Acta*, 1952, **35**, 1942) in " AnalaR " acetic acid (10 ml.) was treated at room temperature with chromium trioxide equivalent to twice that required for the oxidation of one secondary hydroxyl group. After 6 hr. no significant amount of oxidant had been consumed (titration) and the cevagenin orthoacetate diacetate was recovered unchanged (m. p., mixed m. p., and rotation).

Reduction of Cevine Orthoacetate Triacetate with Lithium and Liquid Ammonia.—Cevine orthoacetate triacetate (110 mg.) in dioxan (5 ml.; distilled over sodium) was added to a solution of lithium (80 mg.) in liquid ammonia (20 ml.), and the solution stirred for 10 min. with exclusion of moisture. The excess of lithium was destroyed by ammonium chloride, the ammonia allowed to evaporate, and the residue extracted with water and chloroform. Crystallisation of the chloroform-soluble material from ether–ethanol gave *dihydrocevine orthoacetate*, m. p. 262—264° (decomp.; sintering at 185°), $[\alpha]_D$ +48° (*c*, 0·64) (Found : C, 64·7; H, 8·55; N, 2·35; Ac, 7·7. $C_{29}H_{45}O_8N$ requires C, 65·0; H, 8·45; N, 2·6; Ac, 8·05%). In a second experiment the time of reduction was prolonged to 30 min. without altering the properties of the product, $[\alpha]_D$ +47° (*c*, 1·90). Dihydrocevine orthoacetate did not reduce Fehling's solution.

Dihydrocevine orthoacetate (60 mg.) was heated on the steam-bath for 2 hr. with pyridine (1 ml.) and acetic anhydride (2 ml.). The excess of reagents was removed *in vacuo* and the product crystallised from ether–light petroleum (b. p. 40—60°), to give *dihydrocevine orthoacetate triacetate*, m. p. 277—279°, $[\alpha]_D$ +81° (*c*, 0·84) (Found : C, 63·9; H, 7·85; N, 1·85; Ac, 25·2. $C_{35}H_{51}O_{11}N$ requires C, 63·5; H, 7·75; N, 2·1; Ac, 25·95%). The triacetate (16 mg.) in " AnalaR " acetic acid (2 ml.) 0·05N with respect to chromium trioxide consumed no significant amount of oxidant during 70 min. at room temperature.

Methanolysis of Cevine Triacetate.—The triacetate (Barton and Brooks, *Chem. and Ind.*, 1953, 1366; Barton, Brooks, and Fawcett, *J.*, 1954, 2137) (1·05 g.) in methanol (100 ml.) was left for 8 hr. at room temperature. Water (20 ml.) was added and the solution left overnight. Most of the methanol was removed *in vacuo* and the resulting aqueous suspension was extracted with chloroform. Crystallisation of the product from aqueous acetone afforded *cevine* 3 : 4-

diacetate (500 mg.), m. p. 275—278° (decomp. : sintering at 160°, and resolidifying at 190—200°), $[\alpha]_D$ +14° (*c*, 1·21 in acetone), +28° (*c*, 1·18 in EtOH) (Found : C, 63·05; H, 7·9; N, 2·35; Ac, 14·75. $C_{31}H_{47}O_{10}N$ requires C, 62·7; H, 8·0; N, 2·35; Ac, 14·5%).

Cevine 3 : 4-diacetate (167 mg.) in carbon tetrachloride (25 ml.) was treated with chromium trioxide solution (10 ml.; 0·16N) in 98·5% aqueous acetic acid for 40 min. at room temperature (the appropriate control experiments were run at the same time). Crystallisation of the product from ethanol gave the required *ketone* as prisms, m. p. 279—280° (decomp.), $[\alpha]_D$ +17° (*c*, 1·18), +20° (*c*, 1·12), −20° (*c*, 0·70 in 1 : 1 acetone–chloroform) (Found : C, 62·8; H, 7·35; N, 2·45. $C_{31}H_{45}O_{10}N$ requires C, 62·9; H, 7·65; N, 2·35%).

In a related alcoholysis experiment cevine triacetate (70 mg.) was dissolved in 96% ethanol (5·0 ml.) ($[\alpha]_D$ of solution +31°), triethylamine (12 mg.; 1·1 mols.) in water (0·2 ml.) added, and the solution left at room temperature. After 14 days the rotation had fallen to +25°, unchanged after a further 6 months. Cevine 3 : 4-diacetate (60%), identified by m. p., mixed m. p., and rotation {$[\alpha]_D$ +14° (*c*, 1·00 in acetone)}, was isolated without difficulty from the product. Similarly cevine triacetate (79 mg.) in 96% ethanol (5·0 ml.) containing 0·59N-sulphuric acid (0·40 ml.; 2 equivs.) had $[\alpha]_D$ +35° unchanged during 6 months at room temperature; unchanged cevine triacetate (64%), identified by m. p., mixed m. p., and rotation, $[\alpha]_D$ +21° (*c*, 1·70 in acetone), was recovered.

Lead Tetra-acetate and Periodic Acid Titrations.—The general procedures were as in Part I. The lead tetra-acetate oxidations were carried out in pure acetic acid at concentrations approx. 0·005M with respect to the substrate and approx. 0·015M with respect to lead tetra-acetate.

The periodic acid oxidations were carried out in 1 : 4 ethanol–water at concentrations approx. 0·02M of pure periodic acid and 0·005M with respect to substrate. The amount of periodic acid consumed depended very much upon the pH of the solution. Two different techniques were therefore employed, (*a*) making no addition of sodium hydrogen carbonate and (*b*) adjusting the pH to 7·0 by sodium hydrogen carbonate. Illustrative results are summarised in Table 3. It was eventually discovered that no consumption of oxidant occurred at all until the sodium hydrogen carbonate was added. The following experiment is illustrative. Cevine triacetate (100 mg.) was dissolved in 1 : 4 ethanol–water (10 ml.) made 0·05N with respect to periodic acid. After 10 min. an aliquot portion (1 ml.) was titrated in the usual way and indicated the consumption of 0·95 mol. of periodic acid. The remainder of the solution (9 ml.) was diluted with water and repeatedly extracted with chloroform. Crystallisation from aqueous methanol gave back cevine triacetate (65 mg.), identified by m. p., mixed m. p., and rotation {$[\alpha]_D$ +21° (*c*,0·97 in acetone)}. The recovered material was similarly oxidised with (in the aliquot portion) the apparent consumption of 1 mol. of periodic acid, but the starting material was given back on processing without sodium hydrogen carbonate being added. A similar oxidation, adjusted to pH 6·8 with sodium hydrogen carbonate, did not afford starting material; the uptake of periodic acid after 10 min. was 0·95 mol.

Action of Alkali on the Ketone (XII; R = *Angeloyloxy*) *from Cevadine Orthoacetate Acetate and Related Transformations.*—The ketone (XII; R = angeloyloxy) (500 mg.) in methanol (180 ml.) was treated with 5N-sodium hydroxide (20 ml.) at room temperature (20°) for exactly 2 min. The solution was then acidified with acetic acid and concentrated *in vacuo* to 50 ml. The pH was adjusted to 8 and the solution extracted with chloroform. The product crystallised from methanol, to give the unsaturated *ketone* (XIII; R = angeloyloxy) as needles, m. p. 211—212°, $[\alpha]_D$ +39° (*c*, 1·28), $\lambda_{max.}$ 221 mμ (ε 16,800), $\lambda_{max.}$ (compensated for unsaturated-ester absorption by using an equivalent quantity of cevadine in the alcohol blank) 236 mμ (ε 10,200) (Found : C, 67·55; H, 8·05; angeloyl, 12·45. $C_{32}H_{45}O_8N$ requires C, 67·25; H, 7·95; angeloyl, 14·5%). On more prolonged treatment with alkali the unsaturated ketone was transformed into a further compound having $\lambda_{max.}$ 295 mμ, $E^{1\%}_{1\,cm.}$ 500 and giving a positive ferric chloride test. This substance could only be obtained crystalline as a chloroform solvate of indefinite m. p. and $[\alpha]_D$ +172° (*c*, 1·7), +171° (*c*, 1·13). Satisfactory analytical data could not be obtained.

The unsaturated ketone (XIII; R = angeloyloxy) (see above) (59 mg.) in ethyl acetate (5 ml.) was hydrogenated with 10% palladised charcoal; uptake of hydrogen ceased after 0·92 mol. had been absorbed. Crystallisation of the product from chloroform–light petroleum (b. p. 60—80°) gave the *ketone* (XIII; R = α-methyl-*n*-butyroyloxy) as needles m. p. 224—225°, $[\alpha]_D$ +33° (*c*, 0·89), $\lambda_{max.}$ 238 mμ (ε 12,500) (Found : C, 67·05; H, 8·25; N, 2·7. $C_{32}H_{47}O_8N$ requires C, 67·0; H, 8·25; N, 2·45%).

Oxidation of Dihydrocevadine Orthoacetate Acetate.—Cevadine orthoacetate acetate (1·0 g.) in ethyl acetate (50 ml.) was hydrogenated with 10% palladised charcoal for 40 hr. Crystallisation of the product from aqueous acetone gave *dihydrocevadine orthoacetate acetate*, m. p. 278—279°,

$[\alpha]_D$ +63° (*c*, 2·06), no selective absorption in the ultra-violet (Found: C, 65·05; H, 7·95). $C_{36}H_{53}O_{10}N$ requires C, 65·55; H, 8·1%).

Dihydrocevadine orthoacetate acetate (257 mg.) was oxidised in carbon tetrachloride–acetic acid as in the analogous cases described above (uptake 1·88 equiv.). The product, crystallised from ethanol, gave the required *ketone* (XII; R = α-methyl-*n*-butyroyloxy) (186 mg.) as needles, m. p. 252—253° (decomp.), $[\alpha]_D$ +50° (*c*, 1·15), +50° (*c*, 0·98) (Found: C, 65·6; H, 7·5; N, 2·35. $C_{36}H_{51}O_{10}N$ requires C, 65·75; H, 7·8; N, 2·15%). In a second experiment 1·13 g. (82%) of the ketone, $[\alpha]_D$ +49° (*c*, 1·16), was obtained from 1·37 g. of the parent alcohol.

The above-mentioned ketone (200 mg.) was treated with methanol (80 ml.) and sodium hydroxide solution (5N; 10 ml.), as described in the experiment above. The product was identified as the unsaturated ketone (XIII; R = α-methyl-*n*-butyroyloxy) by m. p., mixed m. p., rotation {$[\alpha]_D$ +32° (*c*, 1·19)}, absorption spectrum, and crystal form.

Rearrangement of Dihydrocevine Orthoacetate to Dihydrocevine iso*Orthoacetate and Related Transformations.*—Dihydrocevine orthoacetate (191 mg.) was dissolved in N-sulphuric acid (5 ml.) and left at room temperature (20°) for 30 min. Isolation of the product by adjustment of the pH to 9 and extraction with chloroform followed by crystallisation from methanol–ether–light petroleum (b. p. 40—60°) afforded *dihydrocevine* iso*orthoacetate* (160 mg.), m. p. 265—266°, $[\alpha]_D$ −14° (*c*, 1·04) (Found: C, 65·25; H, 8·45; N, 2·75; Ac, 6·6. $C_{29}H_{45}O_8N$ requires C, 65·0; H, 8·45; N, 2·6; Ac, 8·0%). The same rearrangement was effected by dissolving dihydrocevine orthoacetate in acetic acid at room temperature and leaving it for 1 hr.

Crude cevagenin orthoacetate, $[\alpha]_D$ *ca.* +23° in EtOH, was prepared without difficulty by following the directions of Stoll and Seebeck (*Helv. Chim. Acta*, 1952, **35**, 1942). A portion (40 mg.) was dissolved in N-sulphuric acid (1 ml.). After 1 min. N-ammonia was added. Extraction with chloroform gave a product, $[\alpha]_D$ −18° (*c*, 1·07 in EtOH), which readily afforded cevagenin orthoacetate, m. p. 180—186°, $[\alpha]_D$ −38° (*c*, 1·04 in EtOH), on crystallisation from acetone (needles) or from methanol (prisms). Repetition of the experiment, but leaving the base in N-sulphuric acid for (*a*) 20 and (*b*) 60 min., gave the same result.

In a comparable experiment cevine orthoacetate (205 mg.) was treated with sulphuric acid for 1 min. in the same way. The crude product was heated at 90° with acetic acid (5 ml.) for 1 hr. (to reclose the opened orthoacetate ring; cf. Part II). Working up gave back starting material, identified by m. p., mixed m. p., and rotation.

The effect of N-sulphuric acid (2 ml.) on cevadine orthoacetate acetate (85 mg.) (20 min. at room temperature) was studied in the same way. After reclosure of the orthoacetate grouping cevadine orthoacetate acetate, identified by m. p., mixed m. p., and rotation, was recovered unchanged.

Cevine 16-*Acetate Perchlorate.*—Cevine 3 : 16-diacetate (170 mg.) in ethyl acetate (2·0 ml.) was treated with a solution of aqueous perchloric acid (72%; 0·1 ml.) in ethyl acetate (1·0 ml.). The solution was evaporated *in vacuo* at room temperature and the residue triturated with ether. The resulting solid was crystallised from hot water, to afford *cevine* 16-*acetate perchlorate* (90 mg.) as plates, m. p. 280—282° (decomp.), $[\alpha]_D$ −5°, −6° (*c*, 1·46 and 0·99 respectively in MeOH) (Found: C, 53·05; H, 7·05; Cl, 5·8; Ac, 6·9. $C_{29}H_{45}O_9N,HClO_4$ requires C, 53·4; H, 7·1; Cl, 5·45; Ac, 6·6%). The constitution of this compound is established by the data given above and by the lead tetra-acetate oxidation results summarised in Table 2.

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