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BIOSYNTHESIS OF THE MONOTERPENE (C_{9-10}) UNIT IN ALKALOIDS

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Abstract—Leucine, intermediates of glycolysis and of the Krebs cycle, and two-carbon compounds labelled at C-1 are poor precursors of the C_{9-10} unit of cephaeline. Although acetate was a specific precursor of a typical steroid sitosterol, and showed significant incorporation into cephaeline, neither it nor glycolic acid labelled at C-2 were specific precursors of the C_{9-10} unit of cephaeline in *Cephaelis acuminata*. Neither was acetate a specific precursor of ajmaline or reserpine in *Rauwolfia serpentina*. Glycine-2-^{14}C was a specific precursor of ajmaline and reserpine in *Rauwolfia serpentina*, and of cephaeline in 4–5-yr-old *Cephaelis acuminata* plants fed in summer, but a poor precursor of β-sitosterol in the latter system. However, neither glycine-2-^{14}C nor glyoxalate-2-^{14}C incorporated activity into the C_{9-10} unit of cephaeline or ipecoside in 2-yr-old plants fed in winter. In all cases where ipecoside or emetine were isolated their activities paralleled that of cephaeline. The results suggest that the monoterpene unit of alkaloids is formed by a pathway different from that utilized for sterols, and that the oxidation state of a two carbon compound may exert an effect on whether it is capable of being a specific precursor of a particular monoterpene unit.

INTRODUCTION

THE MONOTERPENE unit has been characterized and intensively studied in relationship to terpenes, steroids, and carotenoids. Recently a monoterpene unit has also been identified in certain glycosides such as loganin, gentiopicroside, and verbenalin, and in some alkaloids. Most are indole alkaloids such as ibogaine, vindoline, and ajmaline, although the quinoline alkaloid quinine, and the isoquinoline alkaloid cephaeline also contain a C_{9-10} unit.

Terpenoid compounds normally arise from mevalonic acid via geranyl pyrophosphate[1–3] (Fig. 1). Research proving a similar origin for the various C_{9-10} units that occur in monoterpene glycosides and alkaloids has been summarized in several reviews, although acetate is apparently incapable of acting as a specific precursor of such C_{9-10} units.[4–7] Conversely acetate is a specific precursor for steroids, carotenoids, and many other terpenes in a well described pathway.[2,3] However, lack of good incorporation of acetate, particularly into monoterpenes, has led to speculation that biosynthetic sources of such compounds other than acetate might exist.[8] Hence an investigation of possible sources of the monoterpene unit in alkaloids was undertaken. Preliminary accounts of some aspects of this work have been published.[9–11]

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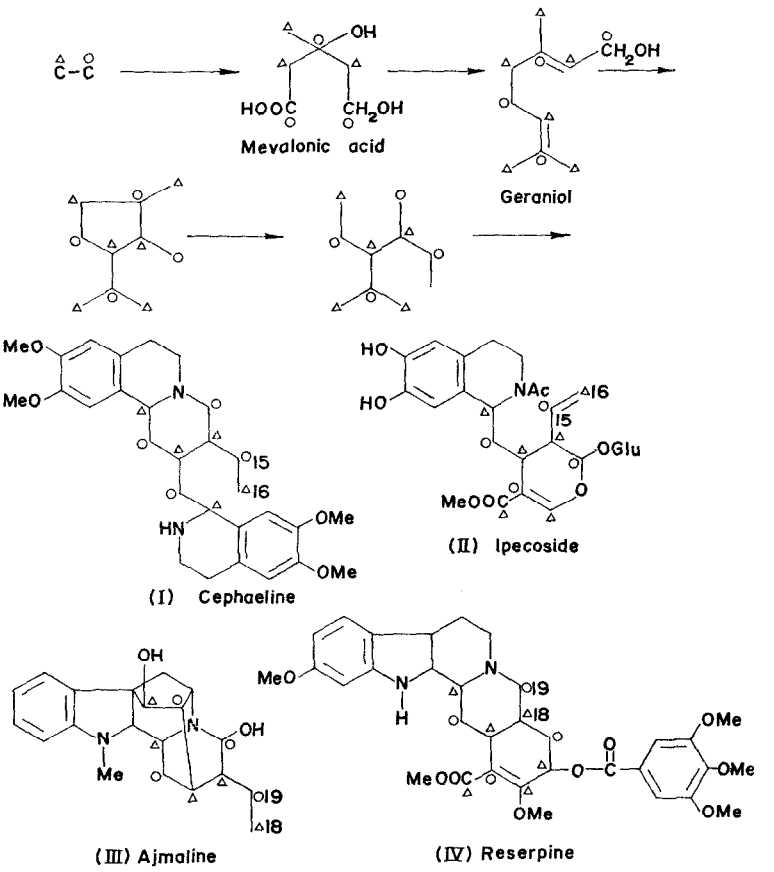

FIG. 1. THEORETICAL INCORPORATION OF LABEL FROM TWO CARBON COMPOUNDS INTO THE C_{9-10} UNIT OF ALKALOIDS.

RESULTS

Specific activities of materials isolated from feeding a variety of radioactive compounds to *Cephaelis acuminata* are summarized in Table 1. Normally the plants were 4–5-yr-old and were fed in early summer under conditions of active growth, but in three cases (Expts. 15–17) the plants were only 2-yr-old and were fed in winter. In cases where emetine or ipecoside (II) were isolated their activities were very similar to that of cephaeline (I).

The incorporation of tyrosine-3-^{14}C (Expt. 1), a known precursor of cephaeline, demonstrated that active synthesis of alkaloids occurred in the system used. However, the low incorporation of radioactive succinate, glycerol, or pyruvate (Expts. 2–6) suggested that neither intermediates in glycolysis, nor intermediates of the Krebs cycle were involved in a major way in the formation of the C_{9-10} unit in cephaeline. Similarly a complete lack of incorporation of leucine (Expt. 7) showed that it was probably not a precursor of this unit.

Two-carbon compounds are not significant precursors of the C_{9-10} unit when they are labelled at C-1 (Expts. 8, 9, 11, 13, 18). This is in agreement with other findings.[6,12–14]

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TABLE 1. RESULTS OF FEEDING OF LABELLED COMPOUNDS TO *Cephaelis acuminata**

Expt. No.	Precursor	Activity fed (μc)	Cephaeline isolated (mg)	Cephaeline† specific activity	Cephaeline per cent incorporation	Emetine† specific activity	Ipecoside† specific activity	Sitosterol specific activity
1	D,L-Tyrosine-3-^{14}C	100	10	130	0·0064	110	N‡	N‡
2	Succinic acid-1,4-$^{14}C_2$	100	21	0	0·0	0	N	N
3	Succinic acid-2,3-$^{14}C_2$	100	25	5·5	0·0006	2·0	N	N
4	Glycerol-1,3-$^{14}C_2$	100	20	4·8	0·0004	4·5	N	N
5	Sodium pyruvate-2-^{14}C	100	10	2·7	0·0002	N	2·2	N
6	Sodium pyruvate-3-^{14}C	100	12	3·8	0·0002	N	4·5	N
7	L-Leucine-U-^{14}C-4,5-$^{3}H_2$	300	15	0	0·0	0	N	N
8§	Sodium acetate-1-^{14}C	250	21	0	0·0	0	N	N
9‖	Sodium acetate-1-^{14}C	250	60	1·4	0·0001	1·8	N	N
10	Sodium acetate-2-^{14}C	250	60	7·7	0·0008	8·5	N	N
11	Sodium glycolate-1-^{14}C	100	97	5·5	0·0025	1·9	N	N
12	Calcium glycolate-2-^{14}C	100	159	28	0·0216	N	N	N
13	Glycine-1-^{14}C	100	10	5·8	0·0003	N	N	N
14	Glycine-2-^{14}C	100	80	57	0·022	8·5	N	N
15¶	Glycine-2-^{14}C	500	200	61	0·012	52	82	N
16¶	Sodium glyoxalate-2-^{14}C	100	9	7·4	0·0003	N	N	N
17¶	Sodium glyoxalate-2-^{14}	200	60	42	0·0061	N	62	N
18	Sodium acetate-1-^{14}C	125	20	4·4	0·0003	N	N	3·9
19**	Sodium acetate-1-^{14}C	125	N	N	N	N	N	15
20	Sodium acetate-2-^{14}C	125	7	1·6	0·0001	N	N	18
21**	Sodium acetate-2-^{14}C	125	12	1·7	0·0001	N	N	92
22	Glycine-2-^{14}C	125	20	100	0·0077	N	N	9·9
23**	Glycine-2-^{14}C	125	12	8·7	0·0004	N	N	N

* Feedings were to 4 plants, 4–5-yr-old, for 10 days in early summer, except where otherwise indicated. † Specific activity figures are counts/min per millimole $\times 10^{-3}$. ‡ N means not isolated. § Fed for 2 days. ‖ Fed for 4 days. ¶ Fed to 2-yr-old plants in winter. ** Fed for 21 days.

In order to investigate the ability of two-carbon compounds at different oxidation states to act as precursors of the C_{9-10} unit acetate, glycolate, glyoxalate, and glycine were fed to *Cephaelis acuminata* under varying conditions (Expts. 8–23), and acetate and glycine were fed to *Rauwolfia serpentina* (Table 2).

TABLE 2. RESULTS OF FEEDING OF LABELLED COMPOUNDS TO *Rauwolfia serpentina**

Expt. No.	Precursor	Activity fed (μc)	Ajmaline† specific activity	Reserpine† specific activity
24	Sodium acetate-2-^{14}C	50	12	6
25	Glycine-2-^{14}C	100	52	13

* Feedings were to 1 plant, 1·5-yr-old, for 7 days in early summer.
† Specific activity figures are counts/min per millimole $\times 10^{-3}$.

Alkaloids and sitosterol isolatable from all sets of experiments in sufficient quantity with sufficient activity were degraded to determine the extent of specificity of incorporation. The results of these degradations are summarized in Table 3.

Acetate-2-^{14}C was a poor precursor of cephaeline (Expts. 10, 20, 21), and moreover showed complete randomization of label between C-15 and C-16 (Expt. 10); although if these figures are representative of the carbons in the C_{9-10} unit, acetate was incorporated into this unit to a greater extent than into the rest of the molecule. Similarly acetate-2-^{14}C was a poor precursor of ajmaline (III) and reserpine (IV) (Expt. 24), and degradation showed definitely that incorporation was not specific to the C_{9-10} unit. Rather the results

TABLE 3. ACTIVITY DISTRIBUTIONS ON COMPOUNDS DEGRADED

Expt. No.	Precursor	Compound*	Per cent activity of compound in Sodium acetate	*N*-Methyl benzamide	Barium carbonate
10	Sodium acetate-2-^{14}C	Cephaeline	13·0	7·0	6·3
12	Sodium glycolate-2-^{14}C	Cephaeline	2·5	1·3	1·2
14	Glycine-2-^{14}C	Cephaeline	16·0	16·0	0·0
22	Glycine-2-^{14}C	Cephaeline	18·0	17·0	0·7
21	Sodium acetate-2-^{14}C	β-Sitosterol	7·2	N†	N†
15	Glycine-2-^{14}C	Cephaeline	1·2	N	N
15	Glycine-2-^{14}C	Ipecoside	0·6	N	N
17	Sodium glyoxalate-2-^{14}C	Cephaeline	0·4	N	N
17	Sodium glyoxalate-2-^{14}C	Ipecoside	2·3	N	N
24	Sodium acetate-2-^{14}C	Ajmaline	9·5	N	N
25	Glycine-2-^{14}C	Ajmaline	15·5	15·5	0·0
			Reserpic acid hydrochloride	3,4,5-Trimethoxybenzoic acid	
24	Sodium acetate-2-^{14}C	Reserpine	59·5	31·8	
25	Glycine-2-^{14}C	Reserpine	84·0	15·0	

* Activity value taken as 100%.
† N means not determined.

are in agreement with complete randomization throughout the whole of each molecule. Hence, although C-2 of acetate can be utilized to a significant extent to produce the entities responsible for the biosynthesis of the C_{9-10} unit, it does not do so specifically. For the sake of comparison β-sitosterol was isolated from *Cephaelis acuminata* (Expts. 18–21). When acetate-2-^{14}C was fed, the sitosterol showed much higher incorporation than did the cephaeline. Moreover Kuhn–Roth degradation of sitosterol (Expt. 21) gave sodium acetate containing 7·2% of the activity of the sitosterol, compared to the expected value of 6·6% if it is biosynthesized in the normal manner.[1] Similar results have been obtained with *Rauwolfia serpentina*.[15]

Glycolate-2-^{14}C showed some incorporation into cephaeline (Expt. 12), but incorporation into C-15 and C-16 was random and very low.

In two cases (Expts. 14, 22) glycine-2-^{14}C showed good incorporation into cephaeline. Furthermore C-16 contained 16–18% of the activity, whereas C-15 was inactive. These figures compare well with a theoretical value of 20% for specific incorporation. Alternatively sitosterol isolated from glycine-2-^{14}C feedings was less active than the corresponding cephaeline, or than sitosterol isolated from acetate-2-^{14}C feedings (Expts. 20–22). Glycine-2-^{14}C also showed incorporation into ajmaline and reserpine. Ajmaline contained 15·5% of the activity in C-18 but none in C-19. Reserpine contained the majority of its activity (84%) in the reserpic acid portion containing the C_{9-10} unit, compared to the more random labelling pattern found when acetate-2-^{14}C was fed (Expts. 24, 25).

These feedings were done on 4–5-yr-old plants in early summer. However, three feedings done on 2-yr-old plants in winter (Expts. 15–17) showed different results. Glycine-2-^{14}C and glyoxalate-2-^{14}C (which is equal to glycine by transamination) both showed some incorporation into both cephaeline and ipecoside. In neither case, however, did C-15 or C-16 of either cephaeline or ipecoside contain any significant portion of the activity.

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DISCUSSION

The present results confirm the lack of specificity of acetate as a precursor of the C_{9-10} unit of cephaeline, at a time when acetate is acting as a specific precursor of β-sitosterol in a normal fashion in the same plant. Leucine has also been described as a biosynthetic source of mevalonate,[16] but was not incorporated into cephaeline (Expt. 7). Similar findings have also been obtained in another laboratory.[17]

Glycolate, glyoxalate and glycine can be precursors of terpenes in chloroplasts,[12] and glycine was a more efficient precursor of the phytol moiety of chlorophylls a and b than was acetate.[13] If any of these two-carbon compounds were converted specifically to mevalonate and geraniol, and thence to the C_{9-10} unit of alkaloids in a typical manner[6,7] then the pattern of incorporation would be as shown in Fig. 1.

Certainly neither acetate nor glycolate fulfils the requirements of such a two-carbon precursor. However, our results suggest that glycine can be such a precursor, both for cephaeline in *Cephaelis acuminata* (Expts. 14, 22), and for ajmaline and reserpine in *Rauwolfia serpentina* (Expt. 25). Glycine-2-^{14}C labelled C-16 but not C-15 of cephaeline, and C-18 but not C-19 of ajmaline; and labelled reserpine predominantly in the reserpic acid portion which contains the C_{9-10} unit. At the same time glycine seemed to be much better able to be incorporated into cephaeline than into sitosterol, whereas acetate was incorporated preferentially into sitosterol (Expts. 20–22). Normal metabolism of glycine-2-^{14}C should divert some activity to one-carbon units, and hence to the methoxy groups of the alkaloids. This might explain the values of 15–18% found in appropriate carbon atoms rather than the theoretically expected 20% for specific incorporation. Glycine might also (by conversion to serine) label the side chain of the tryptophan moiety of ajmaline and reserpine.

Marekov *et al.*[18] have found that glycine-2-^{14}C is specifically incorporated into gentianine in *Gentiana asclepiadae*. However, Groger *et al.*[17] found no specific incorporation of either glycine-2-^{14}C or glyoxalate-2-^{14}C into the C_{9-10} unit of vindoline in *Catharanthus roseus*, and Kutney *et al.*[14] found that in *Vinca rosea* glycine-2-^{14}C activity appeared mainly in the tryptophan portion of ajmalicine, with lesser amounts in the carbomethoxy group. The latter results parallel our feedings on 2-yr-old *Cephaelis acuminata* plants in winter, in which glycine-2-^{14}C and glyoxalate-2-^{14}C both showed some incorporation but delivered essentially no activity to C-15 or C-16 of either cephaeline or ipecoside (Expts. 15–17).

The biosynthesis of the C_{9-10} unit of alkaloids is not a simple operation, and may vary with the oxidation state of the two carbon unit fed, or with various other outside influences. More extensive information is required, and the problem is being actively pursued.

EXPERIMENTAL

Materials. Radioactive chemicals were obtained from New England Nuclear Corporation, Boston, Mass. except for sodium glycolate-1-^{14}C and calcium glycolate-2-^{14}C which were purchased from International Chemical and Nuclear Corporation, City of Industry, California, and sodium glyoxalate-2-^{14}C, sodium pyruvate-2-^{14}C, and sodium pyruvate-3-^{14}C which were purchased from the Radiochemical Centre, Amersham, England. Radioactive samples were counted to within 3% error by means of a Beckman Lowbeta counter (background 0·55–0·75 counts/min). Chemicals used were commercially available except for ipecoside which was a gift from Prof. A. R. Battersby, Cambridge, England.

Plants. Live *Cephaelis acuminata* (Karsten) roots were supplied by Mr. J. B. Kinloch, San Carlos, R.S.J.,

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Nicaragua. The roots were potted and kept in a greenhouse (16 hr/day photoperiod), in a wooden frame chamber (4 × 1 × 1 m), covered on all sides with heavy polyethylene to form a closed system. The chamber was protected from direct sunlight by thick brown paper on the top and three sides. A constant source of water (20-l. glass tank) was maintained at 75–85° by an immersion heater and variable transformer. Evaporation of the hot water maintained conditions similar to the plants natural habitat of a dense humid tropical forest (temp. 30–40°; relative humidity 80–85%; heavy shade). In this chamber 100 plants varying in age from 3 months to 6 yr maintained a healthy growth.

Rauwolfia serpentina (Benth) seeds were obtained from the Himalayan Drug and Research Co., Deharodoon, India, and grown in a sand-soil mixture under normal greenhouse conditions, although the germination rate was only 10 per cent.

Plants were fed radioactive compounds by a wick-feeding method under normal greenhouse growing conditions, and allowed to continue growth for an appropriate length of time. It was ascertained that in all feedings at least 98% of the radioactive material was taken into the plant. Details of feedings appear in Tables 1 and 2.

Isolations from Cephaelis acuminata.* Oven dried chopped roots (11 g) were extracted for 24 hr periods with 0·1 M H_2SO_4 (3 × 50 ml), and the residual root material was retained for the isolation of ipecoside and of β-sitosterol. The combined extracts were reduced to 100 ml, the pH was raised to 9 with NH_4OH, and the solution was extracted with trichloroethylene (5 × 100 ml). The combined extracts were filtered through Celite, reduced to 50 ml, and extracted with 1 N HCl (4 × 25 ml). The combined extracts were reduced to 30 ml and the pH raised to 8 with K_2CO_3. Alkaloids appeared as a precipitate and were extracted with Et_2O (4 × 50 ml). The combined extracts were reduced to 75 ml and extracted four times with 10% aq. KOH (10 ml). After each extraction the basic layer was rapidly re-extracted with Et_2O (25 ml). The combined basic layers, which contained cephaeline, were acidified to pH 2 with HCl and stored in the cold.

The combined Et_2O extracts were washed free of base, dried, filtered, and 1 N HCl (1·5 ml) was added. The Et_2O was removed by evaporation and K_2CO_3 added. The solid was isolated to give a typical yield of 5 mg of emetine m.p. 72–74°.

The acidic solution containing cephaeline was reduced to 5 ml, the pH was raised to 8 with K_2CO_3, and the precipitate was extracted with Et_2O (4 × 25 ml). The combined extracts were washed free of base, dried, and 1 N HCl (2 ml) was added. Et_2O was removed by evaporation and K_2CO_3 was added. The solid was isolated to give a typical yield of 25 mg of cephaeline m.p. 110–111°. The amounts of cephaeline isolated appear in Table 1.

Roots (100 g) which had been extracted with H_2SO_4 to remove alkaloids were macerated in a blender with saturated Na_2CO_3, and allowed to stand for 5 hr with further solid Na_2CO_3 being added at intervals until the pH remained at 5. The mixture was filtered and the residual roots were extracted three times for 10 hr at 50° with aq. EtOH (3:1; 300 ml). The combined Na_2CO_3 and EtOH extracts were reduced to 200 ml, saturated with NaCl, and extracted 3× $CHCl_3$–EtOH (4:1; 500 ml). The combined extracts were washed with saturated NaCl and evaporated. The residue in MeOH–Et_2O (1:3; 10 ml) was chromatographed on silicic acid (100 mesh; 25 g), eluted with MeOH–Et_2O (1:3; 400 ml) and the eluate evaporated. The residue was washed with benzene, and the insoluble material was taken up in EtOH. Ipecoside precipitated and was recrystallized from MeOH to give a typical yield of 11 mg m.p. 175–176°. In isolations from plants fed radioactive compounds less root material was available, and the active ipecoside was scavenged by the addition of inactive ipecoside after it had been eluted from the column.

Roots (10 g), which had been extracted with H_2SO_4 to remove alkaloids, were washed with saturated Na_2CO_3 and then with H_2O until free of base, dried, ground, and extracted with Et_2O (5× 100 ml). The combined Et_2O extracts were evaporated, EtOH (10 ml) and 50% aq. KOH (3 ml) were added, and the mixture was refluxed for 2 hr. H_2O (10 ml) was added, and the mixture was extracted 5 × light petroleum (60–68°; 50 ml). The combined extracts were washed free of base, dried, filtered and evaporated. Pure inactive β-sitosterol (7 mg) was added to the residue, and recrystallization from EtOH yielded pure sitosterol m.p. 136–138°.

Isolation from Rauwolfia serpentina. Dried whole plant material (10 g) was macerated in a blender with $CHCl_3$ (100 ml), and 3 N NH_4OH (12 ml), allowed to stand for 2 days, filtered through cloth, and washed with $CHCl_3$ and H_2O. The $CHCl_3$ layer was evaporated to 15 ml, and extracted with 1 M KH_2PO_4 (5 × 25 ml) to yield an aq. solution (A) and a $CHCl_3$ solution (B).

Solution (A) was made basic with 15 N NH_4OH, extracted with $CHCl_3$ (5 × 10 ml), and the combined extracts dried and evaporated. The residue in $CHCl_3$ was chromatographed on Merck alumina (activity III; 10 g), washed with $CHCl_3$ (30 ml), and eluted with $CHCl_3$–MeOH (9:1; 50 ml). The eluate was evaporated, the residue dissolved in 10% HOAc (2 ml), and the pH raised to 8 with 15 N NH_4OH. The mixture was extracted 4× Et_2O–MeOH (9:1; 10 ml), and the combined extracts were evaporated. The residue in $CHCl_3$ was chromatographed on Florosil (60–200 mesh; activity 1200F; 6 g), washed with $CHCl_3$ (15 ml), and

* Compounds isolated or produced by degradation were ascertained to be identical in all respects with authentic samples of the appropriate compound. All compounds isolated from feeding experiments were recrystallized to constant activity.

eluted with $CHCl_3$–MeOH (30 ml), and MeOH (10 ml). The eluate was evaporated, and pure inactive ajmaline (15 mg) was added to the residue. Pure ajmaline m.p. 157–158° was isolated by recrystallization from MeOH.

Solution (B) was evaporated to 5 ml, light petroleum (60–68°; 25 ml) was added, and the mixture was extracted with 1 N H_2SO_4–MeOH (2:1; 30 ml). The aqueous layer was filtered, made alkaline with NH_4OH, extracted with $CHCl_3$ (4 × 10 ml), and the combined extracts were dried and evaporated. The residue in $CHCl_3$ was chromatographed on alumina (activity III; 15 g), and eluted with $CHCl_3$ (60 ml). The eluate was evaporated and pure inactive reserpine (20 mg) was added to the residue. Pure reserpine m.p. 265–266° was isolated by recrystallization from acetone–MeOH.

Hydrogenation of ipecoside to dihydroipecoside. Ipecoside (50 mg) and 10% Pd–C (6 mg) in ab. EtOH (5 ml) were stirred under H_2 for 24 hr. Filtration and evaporation yielded dihydroipecoside (46 mg; 92%).

Hydrolysis of reserpine. Reserpine (10 mg) and KOH (300 mg) in MeOH–H_2O (5:1; 6 ml) were refluxed for 1 hr, the solution was made strongly acidic with conc. HCl and evaporated. The residue was dissolved in H_2O (25 ml), extracted with Et_2O (3 × 10 ml), and the combined extracts evaporated. The residue was recrystallized from H_2O to yield 3,4,5-trimethoxybenzoic acid (2 mg; 60%; m.p. 174–175°). The residual aq. solution was evaporated, and the residue heated with MeOH, filtered and evaporated. A little dil. HCl was added, and the resulting solid was recrystallized from MeOH–Et_2O to yield reserpic acid HCl (4 mg; 55%; m.p. 266–268°.)

Kuhn–Roth oxidation of cephaeline, ajmaline and dihydroipecoside. Cephaeline, ajmaline, or dihydroipecoside (50 mg), and CrO_3 (2 g) in 10% H_2SO_4 (4 ml) were refluxed under N_2 at 110° for 6 hr. H_2O (20 ml) was added, and the system was distilled with further addition of H_2O until 100 ml of distillate had been collected. The distillate was titrated to pH 10 with NaOH, evaporated to 15 ml, extracted with Et_2O (4 × 5 ml), and further evaporated. The residue was dissolved in abs. EtOH, filtered and evaporated, and the residue dried at 115° for 12 hr. It was then dissolved in a minimum amount of H_2O, acidified to pH 2 with dil. H_2SO_4, absorbed on dry silicic acid (3 g), and dried under vacuum. A column was prepared from silicic acid (5 g), 0·5 N H_2SO_4 (3·5 ml), and enough $CHCl_3$ to form a slurry. The dried silicic acid was added and the column eluted with *n*-BuOH–$CHCl_3$ (1:49; 90 ml), 10 ml fractions being collected. Fractions 4–9 were combined, H_2O (25 ml) was added, and the mixture was exactly neutralized with NaOH. Evaporation gave a typical yield of 5 mg (50%) of pure Na acetate containing no detectable amount of Na propionate.

Kuhn–Roth oxidation of sitosterol. Sitosterol (20 mg) was boiled gently under N_2 with CrO_3 (1 g) in H_2O (5 ml) for 11 hr, H_3PO_4 (density 1·75; 1 ml) was added and the mixture was distilled with addition of H_2O until 50 ml of distillate had been collected. The distillate was neutralized with NaOH and evaporated. The Na acetate obtained was purified as before to yield the pure compound (10 mg; 51%).

Schmidt reaction on sodium acetate. To Na acetate (20 mg) at 0° under CO_2 free N_2 was added conc. H_2SO_4 (0·8 ml) and NaN_3 (40 mg). The mixture was heated at 90° for 1 hr, and the CO_2 liberated collected as $BaCO_3$ (20 mg; 42%). The residual solution was made basic with 20 N NaOH, H_2O (20 ml) added and the $MeNH_2$ was distilled into 10% HCl (10 ml). After evaporation benzoyl chloride (2 drops) and 20% NaOH (2 ml) were added to the residue which was allowed to stand for 12 hr. The mixture was extracted with Et_2O (4 × 10 ml), the combined extracts evaporated, and the residue recrystallized from light petroleum (60–68°)–Et_2O (4:1) to yield *N*-methylbenzamide (3 mg; 10% m.p. 73–75°).

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Key Word Index—Cephaelis acuminata; Rubiaceae; *Rauwolfia serpentina*; Apocynaceae; biosynthesis; terpene alkaloids.