Biosynthesis of the *Cinchona* Alkaloids: Late Stages of the Pathway

By A. R. BATTERSBY* and R. J. PARRY

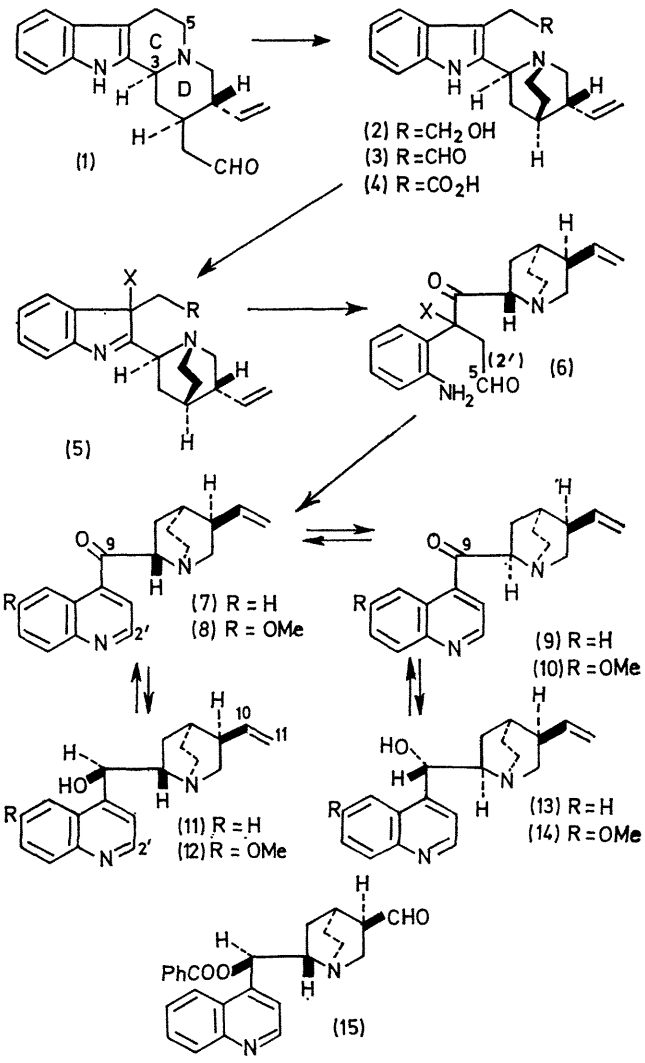
(*University Chemical Laboratory, University of Cambridge, Cambridge* CB2 1EW)

Summary A combination of evidence from isolations and from tracer experiments supports the sequence (**1**) → (**7**) and (**9**) ⇄ (**11**) and (**13**) (and similarly for the methoxylated series) as the pathway to the *Cinchona* alkaloids: the results also point to cinchonaminal (**3**) as being the key intermediate between (**1**) and (**7**).

THE preceding communication[1] traced the biosynthetic pathway to the quinoline alkaloids of *Cinchona* as far as corynantheal (**1**). Ideas[2–4] regarding the subsequent steps have made use of cinchonamine (**2**) or a close relative as an intermediate and our working hypothesis was the sequence (**1**) → (**3**) → (**5**) → (**6**) → (**7**). The ketone (**7**) could then lead, as illustrated, to the main quinoline bases (**11**—**14**). Experiments which support this scheme are now outlined.

An understanding of the processes whereby ring c of corynantheal (**1**) is cleaved requires knowledge of the oxidation level at C-5 of (**1**) as it passes along the biosynthetic pathway to become C-2′ of the quinoline bases [see *e.g.* (**11**)]. This was examined by preparing [1-$^3H_2$]-tryptamine from indolylacetonitrile using borotritiide–cobalt chloride.[5] When the product was administered to *Cinchona ledgeriana* shoots in admixture with [1-^{14}C]tryptamine, the results under Expt. 1 (Table) were obtained. Cinchonidinone (**7**), prepared by oxidation[6] of cinchonidine (**11**), was added to the work-up of Expt. 1 in addition to the three known bases (**11**—**13**). The isolated ketone was purified to constant activity and then was reduced with borohydride to a mixture of cinchonidine (**11**) and cinchonine (**13**); the latter was purified to constant activity. The results from Expt. 1 show (i) that cinchonidinone (**7**) is a natural product, (ii) within experimental error, 50% of the tritium at C-1 of tryptamine [corresponding to C-5 of corynantheal (**1**)] is lost during the formation of the four quinoline systems and it follows that oxidative attack at C-5 of (**1**) is a stereospecific process, and (iii) a carboxylic acid such as (**4**) cannot be a biosynthetic intermediate.

The methoxylated ketones (**8** and **10**) would also be expected to be present in *Cinchona* plants and this was tested by adding quinidinone (**10**) to the alkaloids from



Expt. 1 described in the preceding communication. Re-isolation and rigorous purification afforded active quinidinone and its radiochemical purity was checked by reduction as yield formaldehyde from C-11 which carried 105% of the original molar activity. Labelling was thus proved to be entirely at the expected site.

Tracer experiments on Cinchona ledgeriana

Expt. no.	Precursor	Incorporations (%) Cinchonidinone (**7**)	Cinchonine (**13**)	Cinchonidine(**11**)	Quinine(**12**)
1.	[1-$^3H_2$,1-^{14}C]Tryptamine Ratio ^{3}H: ^{14}C 4·70	0·47; Ratio 2·28 (48% retention ^{3}H)	0·20; Ratio 2·33 (50% retention ^{3}H)	0·12; Ratio 2·45 (52% retention ^{3}H)	0·33; Ratio 2·48 (53% retention ^{3}H)
2.	[11-$^3H_2$]Cinchonidinone(**7**)		0·14	0·03	0·002
3.	[11-$^3H_2$]Cinchonidine(**11**)	0·06			
4.	[*Ar*-^{3}H]Cinchonamine(**2**)		0·001	0·0008	0·0001

described above to give quinine (**12**) and quinidine (**14**). The latter was isolated and finally purified as its phenylcarbamate derivative; its constant activity corresponded to 0·002% incorporation which establishes the presence of quinidinone (**10**) in the plants.

[11-$^3H_2$]Cinchonidinone (**7**) was prepared for incorporation experiments as follows. Treatment of *O*-benzoylcinchonidine [*cf.* (**11**)] with osmium tetroxide–periodate yielded the aldehyde (**15**), m.p. 189—191° which was converted by [*methylene*-$^3H_2$]triphenylphosphine methide into *O*-benzoyl-[11-$^3H_2$]cinchonidine. Specificity of labelling was confirmed by cleaving the vinyl group as before from a diluted aliquot of this product to give formaldehyde which carried 98% of the activity of the starting material. The ester group of the high activity *O*-benzoyl-[11-$^3H_2$ cinchonidine was hydrolysed, and oxidation then afforded [11-$^3H_2$]cinchonidinone (**7**). Expt. 2 showed that this precursor was incorporated into the main *Cinchona* alkaloids. The isolated cinchonine (**13**), as its phenylcarbamyl derivative, was degraded with osmium tetroxide-periodate to

The reversibility of the step (**7**) → (**11**) was studied by feeding [11-$^3H_2$]cinchonidine (**11**) to *C. ledgeriana* shoots and isolating cinchonidinone (**7**), added as carrier. The product was purified by the same reductive sequence used above and the result (Expt. 3) showed that reversal does occur [*i.e.*, (**11**) → (**7**)].

Finally, cinchonamine (**2**) was assessed as a possible precursor of the *Cinchona* alkaloids by *Ar*-labelling (using [^{3}H]trifluoroacetic acid) and administration of the product to the plants. The incorporations (Expt. 4), though not zero, are so low that it is unlikely that cinchonamine (**2**) is on the direct pathway. This result taken in conjunction with Expt. 1 makes it probable that cinchonaminal (**3**) is the key intermediate between corynantheal (**1**) and the 9-keto-quinoline bases (**7**—**10**).

We thank Prof. R. Goutarel (Gif-sur-Yvette) for generous gifts of alkaloids.

(*Received, November 4th*, 1970; *Com.* 1918.)

[1] A. R. Battersby and R. J. Parry, preceding communication.
[2] R. Goutarel, M.-M. Janot, V. Prelog, and W. I. Taylor, *Helv. Chim. Acta*, 1950, **33**, 150.
[3] A. R. Battersby and E. S. Hall, *Chem. Comm.*, 1970, 194.
[4] E. Leete, *Accounts Chem. Res.*, 1969, **2**, 59.
[5] T. Satoh, S. Suzuki, Y. Suzuki, Y. Miyaji, and Z. Imai, *Tetrahedron Letters*, 1969, 4555.
[6] R. B. Woodward, N. L. Wendler, and F. J. Brutschy, *J. Amer. Chem. Soc.*, 1945, **67**, 1425.