Stereochemical Studies concerning the Biosynthesis of Narcotine, Protopine, and Chelidonine

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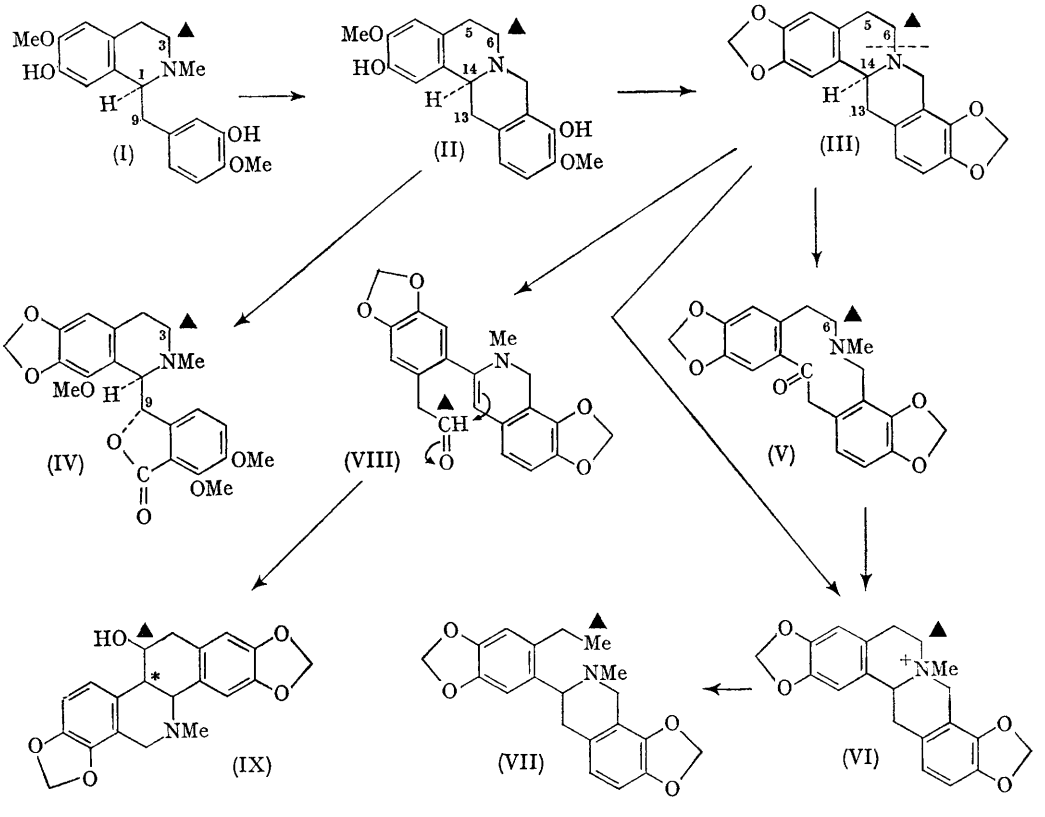
OUR earlier work with multiply-labelled precursors proved[1] that the opium alkaloid narcotine (IV) is biosynthesised from reticuline (as I) and, importantly, that the carbonyl residue is derived without loss of carbon from the *N*-methyl group of (I). This supported the view[2] that phthalideisoquinolines are formed from tetrahydroprotoberberines and scoulerine (as II) was suggested[1] as the precursor. The synthesis[3] of (−)-[6-^{14}C,14-^{3}H]-scoulerine (II) and its enantiomer allowed critical tests in *Papaver somniferum* plants. Experiment 1 (Table) shows that the (−)-isomer, which corresponds[3] to narcotine[4] in absolute configuration, is incorporated well with some tritium loss whereas the (+)-form is virtually ineffective (Expt. 2). Degradation of the narcotine[5] proved 97% of its ^{14}C activity to be located at C-3 (see IV) so demonstrating the protoberberine → phthalideisoquinoline conversion. Scoulerine has recently been isolated from opium.[6] The oxidative process whereby C-13 of (II) becomes the lactonic C-9 in narcotine (IV) was studied with (−)-, and (+)-[3-^{14}C,9-$^{3}H_2$]-reticuline. As expected, the (+)-form (I) was the better precursor (Expts. 3 and 4) and loss of *ca.* 50% of the tritium from C-9 of (I) during its biological conversion into narcotine is in accord with a stereospecific oxidation at C-13 of (−)-scoulerine (II) or a close relative.

The biosynthesis of protopine (V) in *Dicentra* plants has been shown[7] to involve (+)-reticuline (I). That (−)-scoulerine (II) is a further intermediate in *Chelidonium majus* is established by Expts. 1, 2, 7, and 8. The ^{3}H values recorded should be compared with those for stylopine and are in agreement with the conversion (II) → (III) → (V). The exact mechanism of the second stage requires further study. Again only (−)-scoulerine acted as an effective precursor of stylopine (III) and protopine (V). Conversion of the protopine *via* (VI) into (VII) followed by Kuhn–Roth oxidation gave acetic acid which was

TABLE

Expt. No.	Precursor	(±)-Stylopine[d] (as III) Incorp.[a]	(±)-Stylopine[d] (as III) %[b] Loss or gain ^3H	Narcotine (IV) Incorp.[a]	Narcotine (IV) %[b] Loss or gain ^3H	Protopine (V) Incorp.[a]	Protopine (V) %[b] Loss or gain ^3H	Chelidonine (IX) Incorp.[a]	Chelidonine (IX) %[b] Loss or gain ^3H
1	(−)-[6-^{14}C,14-^{3}H]-(II)	0·28	+10	2·3	−13	0·92	−100	0·61	−100
2	(+)-[6-^{14}C,14-^{3}H]-(II)	0·013	c	0·02	c	0·04	c	0·014	c
3	(+)-[3-^{14}C,9-$^3H_2$]-(I)	0·53	−4	0·09	−46	—	—	0·58	−18
4	(−)-[3-^{14}C,9-$^3H_2$]-(I)	0·02	−2	0·03	−55	—	—	0·03	−16
5	(±)-[6-^{14}C,5-$^3H_2$]-(II)	0·3	+10	—	—	—	—	1·2	+23
6	(±)-[6-^{14}C,6-$^3H_2$]-(II)	0·2	c	—	—	—	—	1·2	−38
7	(−)-[6-^{14}C,6-$^3H_2$]-(II)	0·18	+9	—	—	0·72	+9	0·81	−37
8	(+)-[6-^{14}C,6-$^3H_2$]-(II)	0·009	c	—	—	0·01	c	0·01	c

[a] Incorporations are based upon ^{14}C; comparable feeding conditions were used for each enantiomeric pair.
[b] Calculated relative to the ^{14}C-label; the figures show the % change in ^{3}H: ^{14}C ratio from that in the precursor.
[c] Not examined.
[d] Stylopine is present as a partial racemate containing an excess of the (−)-form. The specific activities of the (−)- and (±)-forms proved that little labelling of the (+)-form occurred over the feeding period.



degraded by Schmidt's method to methylamine which contained all the original activity. Protopine (V) was thus proved to be labelled specifically at C-6. Stylopine (III) was similarly degraded with the same result.

Attention is drawn to the small rise in ^{3}H:^{14}C ratio (*ca.* 10%) for those biological conversions not involving the ^{3}H-labelled site *e.g.*, stylopine in Expts. 1 and 7 and protopine in Expt. 7. This effect has been observed in other cases and will be

discussed in our full Paper; it is of importance in the sequel for chelidonine.

The late stages leading to chelidonine (IX) in *C. majus* were shown in this laboratory[8] to be (I) → (II) → (III) → (IX) and the intervention of stylopine (III) has been confirmed.[9] These results are in agreement with earlier suggestions that chelidonine is derived in some way from the tetrahydroprotoberberine skeleton.[10] Support for a mechanism *via* (VIII) has now been obtained by examining the fate of the hydrogen atoms attached to C-5, -6, -13, and -14 of (−)-stylopine (III) during its biological conversion into chelidonine (IX). (−)-Scoulerine, and in two experiments reticuline (I), labelled with ^3H at the positions corresponding to C-5, -6, -13, and -14 of stylopine (III) were used for these studies. For each enantiomeric pair, the substance corresponding in absolute configuration to (−)-stylopine (III) was by far the more effective precursor of chelidonine. Expt. 1 shows that the hydrogen atom at C-14 is lost completely. Expt. 5 indicates that C-5 is not involved in the stylopine → chelidonine transformation but C-6 is clearly affected (Expts. 6 and 7). The losses of ^3H recorded in the Table are calculated from the $^3H/^{14}C$ ratio in the original precursor and when account is taken of the general rise in this ratio along the biosynthetic pathway, ^3H-loss from C-6 becomes close to that expected (50%) for a stereospecific oxidation which does not involve an isotope effect. In contrast, Expt. 3 shows that the $^3H:^{14}C$ ratio in chelidonine is only 18% below that of the precursor proving a small ^3H-loss from C-13. Even allowing for the general rise in ^3H level, this loss is well below 50% and suggests for C-13 a nonstereospecific removal of hydrogen involving an isotope effect. Complete loss of ^3H when chelidonine from Expt. 3 was converted into the methine by Hofmann degradation established specific ^3H-labelling at the starred position in the alkaloid (IX) and the illustrated location of the ^{14}C label was proved as earlier.[8] Stereospecific generation of a C-14/N double-bond in (III) followed by isomerisation to the C-13/C-14 enamine would explain these results which contrast with those indicating a sterically controlled attack at C-13 (see II) in narcotine biosynthesis.

Scoulerine labelled at C-6 and C-13 with ^3H in known absolute configuration is being used in current work to discover the stereochemistry of the oxidative processes.

(*Received, May 10th*, 1967; *Com.* 451.)

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