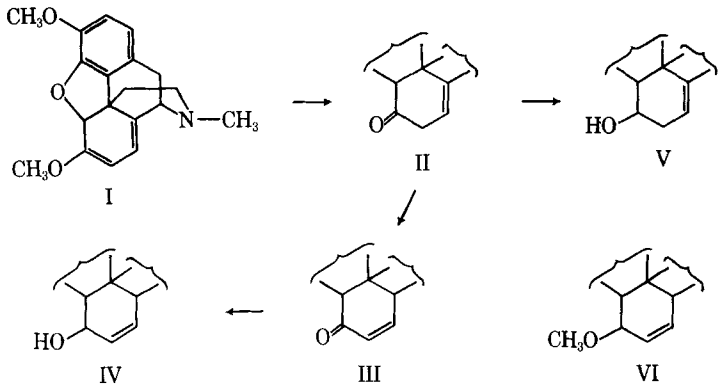
Biosynthetic Conversion of Thebaine to Codeine and Role of Codeine Methyl Ether

Sir:

The mechanism by which thebaine (I) is converted to codeine in the opium poppy has been postulated to involve demethylation to the ketone neopinone (II), rearrangement to codeinone (III), followed by reduction to codeine (IV) (1–3). This scheme also explains the presence in the plant of the alkaloid neopine (V) as a reduction product of neopinone. In 1965 codeine methyl ether (VI) was isolated from the crude mother liquor obtained during the commercial production of morphine (4).



The suggestion was made that this substance might conceivably be a biosynthetic intermediate between thebaine and codeine if the reduction step preceded demethylation. It was therefore decided to test these hypotheses by feeding experiments with radioactively labeled codeinone and codeine methyl ether and by demonstrating whether these two substances exist as genuine alkaloids in the fresh plant. This investigation had been completed when Blaschke, Parker, and Rapoport (5) described experiments with generally labeled materials of a similar nature and with similar results. Our work with specifically labeled codeinone, which provides independent evidence for the role of this compound in the biosynethetic conversion of thebaine to codeine, is published elsewhere (6). However, we want to report here on our experiments with codeine methyl ether and the conclusions which may be drawn from these.

Codeine *N*-oxide (3-$^{14}CH_3$) was prepared as described by Chang *et al.* (7). It was methylated with dimethyl sulfate, and reduced with sulfur dioxide to codeine (3-$^{14}CH_3$) methyl ether which was purified by preparative thin-layer chromatography and crystallization from ether–petroleum ether, m.p. 142° (micro K), specific activity 1.35 mc./mmole. The substance was shown to be chromatographically pure by thin-layer and gas chromatography. Autoradiograms (7 days' exposure) from thin-layer chromatograms with two solvent systems (8) revealed only one radioactive spot corresponding to codeine methyl ether. In July 1965, 56 mg. (0.241 mc.) of this substance was administered to 5 opium poppies by a technique described before (9). The plants were harvested 3 days later, extracted with methanol, and the nonphenolic alkaloid fraction isolated. An autoradiogram of this fraction showed radioactive codeine and codeine methyl ether, while thebaine was inactive. Preparative thin-layer chromatography on silica gel GF (benzene–ethanol, 8 : 2, triple development) gave codeine (41 mg.), codeine methyl ether (48 mg.), and thebaine (56 mg.). Codeine was purified by repeated crystallization, first as the picrate, then as the free base, until the activity remained constant (liquid scintillation counting). Total incorporation of radioactivity from the methyl ether was 2.5%. A Zeisel *O*-demethylation showed that all activity resided in the *O*-methyl group. Although these results were encouraging, it was felt that the amount of precursor which had been fed was too large for the number of plants, possibly resulting in a swamping of the biosynthetic pool. Second, the feeding time of 3 days might have been too short for efficient incorporation since most of the methyl ether was recovered unchanged. The feeding experiments were, therefore, repeated in May 1966 with 5 mg. of labeled codeine methyl ether for 10 plants and a 7-day feeding period. This resulted in an incorporation into codeine of 15.8% of the total radioactivity. Thus, it is clear that

the opium poppy is capable of demethylating codeine methyl ether. However, this did not prove that the methyl ether is a required intermediate. It would be necessary to show (*a*) that this substance is present in the fresh plant, and (*b*) that it is formed directly from thebaine. The biosynthetic pathway leading from reticuline to thebaine is now well established (10–12). Radioactively labeled (±)-reticuline-3-^{14}C (0.045 mc.) (10) was administered to 9 opium poppies. The plants were harvested 2 weeks later, and 100 mg. of radioinactive codeine methyl ether was added to the plant mash during extraction. It was recovered by isolation and separation of the nonphenolic alkaloids and was found to be practically inactive (0.0004% incorporation). This suggested that codeine methyl ether is not produced in the plant by way of thebaine, nor is it derived from codeine. The latter point was also confirmed by addition of the unlabeled methyl ether to an extract from 20 opium poppies which had been fed 2,6-^{3}H-codeine (0.108 mc.) (6). Again the isolated codeine methyl ether was essentially inactive (0.0003% incorporation). We, therefore, conclude that codeine methyl ether is not a genuine opium alkaloid, at least not in the varieties of *P. somniferum* which we have used.

When the isolation of codeine methyl ether was reported, the possibility of this substance being an artifact was considered (4). During large-scale production of morphine, reducing agents such as sulfur dioxide, bisulfite, or hydrosulfite are usually added as antioxidants. It now appears reasonable to believe that the small amounts of codeine methyl ether present in the mother liquors were actually produced by reduction of thebaine during the isolation and purification of morphine.

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