Phenol Oxidation and Biosynthesis. Part XII.[1] Stereochemical Studies Related to the Biosynthesis of the Morphine Alkaloids

By D. H. R. Barton, D. S. Bhakuni, R. James, and G. W. Kirby, Imperial College, London S.W.7

Ozonolysis of tritiated salutaridinols-I and -II has afforded glyceric acids whose absolute configurations have been determined by the isotope dilution method. In this way the stereochemistry of salutaridinol-I, the precursor of the morphine alkaloids, has been defined unambiguously.

Oxidation of tritiated (+)- and (−)-reticuline, followed by isotope dilution analysis, has confirmed the configurational relationship between benzylisoquinoline and morphine alkaloids. The results obtained amount to a long sought total synthesis of morphine from reticuline following the biogenetic route.

The configurational relationship between morphine and benzylisoquinoline alkaloids has been further confirmed by the reduction of salutaridine to give, after appropriate methylation, (−)-laudanosine. The bond broken in this reduction is the bond formed in the biogenetic oxidative cyclisation of (−)-reticuline.

In an earlier Communication [2] we showed that reduction of the morphine precursor, salutaridine (I), with sodium borohydride gave two alcohols (II), named salutaridinol-I and -II. Salutaridinol-I, but not its epimer, was found to be a biological precursor of thebaine (III) in *Papaver somniferum*.[3] We now report experiments leading to the absolute (and hence, also, the relative) stereochemistry (IV) for the hydroxyl group in salutaridinol-I.

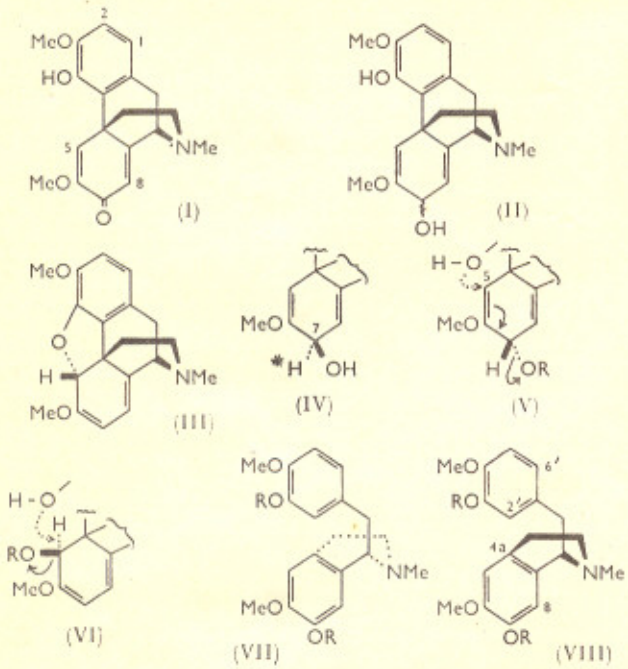
In principle, ozonolysis of either salutaridinol, followed by reduction of the ozonide and hydrolysis of the reaction products, should give optically active glyceric acid. D-(—)-Glyceric acid would be obtained from the alcohol of configuration (IV). In practice the yields of glyceric acid from this degradation were inconveniently low (*ca.* 1%). A radio-dilution method, therefore, was employed to determine the configuration. Salutaridine was reduced with sodium [^{3}H]borohydride to give the two salutaridinols labelled at C-7 [see asterisk in (IV)]. [^{3}H]Salutaridinol-I was ozonised at −70° in ethanol and the reaction mixture hydrogenated directly over Adams catalyst. The total product was hydrolysed with alkali and then diluted with non-radioactive D-glyceric acid. The acid was converted into its *p*-bromophenacyl

[1] Part XI, D. H. R. Barton, G. W. Kirby, and A. Wiechers, *J. Chem. Soc.* (*C*), 1966, 2313. The content of the present Paper has been partly presented in preliminary form (D. H. R. Barton, G. W. Kirby, W. Steglich, and G. M. Thomas, *Proc. Chem. Soc.*,

[2] D. H. R. Barton, G. W. Kirby, W. Steglich, and G. M. Thomas, *Proc. Chem. Soc.*, 1963, 203.

[3] D. H. R. Barton, G. W. Kirby, W. Steglich, G. M. Thomas, A. R. Battersby, T. A. Dobson, and H. Ramuz, *J. Chem. Soc.*,

derivative [4] which was purified by chromatography and crystallisation. This derivative retained its radioactivity after repeated recrystallisation, showing that it contained *p*-bromophenacyl D-[^{3}H]glycerate. Salutaridinol-I must, therefore, have the configuration (IV).



Racemisation at any stage during isolation would result in loss of tritium and thus could not invalidate the conclusion drawn. The activity of the end product was compared with that of the starting alcohol to give the yield of glyceric acid formed during degradation, allowance being made for loss of material during preparation of the *p*-bromophenacyl derivative (see Table). In agreement with this assignment of configuration to salutaridinol-I, a similar degradation of salutaridinol-II gave non-radioactive *p*-bromophenacyl D-glycerate. Moreover, a corresponding pair of experiments using L-(+)-glyceric acid for dilution gave the expected, opposite result, radioactive L-glyceric acid being produced from salutaridinol-II and not from its epimer. The preparation of calcium D-glycerate from D-mannitol, and of calcium L-glycerate from L-serine, is recorded in the Experimental section.

The biological conversion of salutaridinol-I into thebaine formally requires loss of the elements of water and might involve a phosphorylated intermediate.[5] If cyclisation involved a one-step, S_N2', displacement of phosphate, then salutaridinol-II [see (V)] and not its epimer would have the appropriate configuration [6] at C-7. It seems likely, therefore, that an additional step must be involved in the biochemical process. Two

Degradation of the salutaridinols to glyceric acid

Salutaridinol	I	II	I	II
Configuration of glyceric acid	D	D	L	L
Yield on esterification (%) *	28	16	9	24
Yield of glyceric acid (%) †	0·80	0·06	<0·04	0·78

* Esterified with *p*-bromophenacyl bromide. † Calculated by isotope dilution analysis.

possibilities can be considered. First, direct displacement, at C-7, in a salutaridinol-I derivative, by an enzyme functional group would give an enzyme-bound intermediate with the inverted configuration required for S_N2' ring closure. Second, a preliminary allylic (7 ⟶ 5) rearrangement would give an isomer (VI) having the correct configuration for S_N2 displacement at C-5.

The absolute configuration [7] of (+)-laudanosine (VII; R = Me) and of the morphine alkaloids [8] has been established by chemical and *X*-ray crystallographic means. (−)-Reticuline (VIII; R = H) and (+)-norlaudanosoline [trisnor- (VIII)] therefore correspond in absolute configuration to thebaine (III). Battersby and his colleagues [9] have however observed that, unexpectedly, (−)-norlaudanosoline is incorporated into morphine, in *Papaver somniferum*, more efficiently than its antipode. It seemed that a direct stereochemical correlation of the benzylisoquinoline and morphine alkaloids would be worthwhile. This we have done in two ways, both of which support the original configurational assignments.

Several unsuccessful attempts have been made to prepare (±)-salutaridine (I) by chemical oxidation of reticuline (VII, VIII; R = H).[10] The corresponding biochemical transformation is well established.[3] Once supplies of (+)-salutaridine became available we were able to study critically, by radiochemical means, its synthesis from reticuline.[2] (±)-Reticuline was labelled in the aromatic rings by heating in acidic[^{3}H] water. A parallel experiment in deuterium oxide showed (n.m.r. control) that all the aryl protons, and no others, exchanged with tritium under these conditions. (±)-[^{3}H]Reticuline was oxidised by a variety of reagents and the crude reaction mixture diluted with non-radioactive (+)-salutaridine. The salutaridine was recovered and rigorously purified by chromatography and crystallisation to constant specific activity. A further check on the radiochemical purity was made by conversion into thebaine *via* the salutaridinols. The final specific activity was used to calculate the yield of (+)-salutaridine formed in the oxidation step, allowance being made for the loss of tritium during oxidative cyclisation.

[4] See G. Büchi, L. Crombie, P. J. Godin, J. S Kaltenbronn, K. S. Siddalingaiah, and D. A. Whiting, *J. Chem. Soc.*, 1961, 2843.

[5] A. R. Battersby, Tilden Lecture, *Proc. Chem. Soc.*, 1963, 189.

[6] Cf. G. Stork, "The Alkaloids," ed. R. H. F. Manske, Academic Press, New York, 1960, vol. VI, p. 227; M. Gates and M. S. Shepard, *J. Amer. Chem. Soc.*, 1962, **84**, 4125.

[7] H. Corrodi and E. Hardegger, *Helv. Chim. Acta*, 1956, **39**, 889.

[8] J. Kalvoda, P. Buchschacher, and O. Jeger, *Helv. Chim. Acta*, 1955, **38**, 1849; G. Kartha, F. R. Ahmed, and W. H. Barnes, *Acta Cryst.*, 1962, **15**, 326.

[9] A. R. Battersby, D. M. Foulkes, and (in part) R. Binks, *J. Chem. Soc.*, 1965, 3323.

[10] W. Wan-Chiu Chan and P. Maitland, *J. Chem. Soc.* (*C*), 1966, 753, and references there cited.

(±)-Reticuline would, of course, also give (−)-salutaridine, which would be lost during repeated crystallisation of the diluted material. The highest yield of (+)-salutaridine (0·015%, corresponding to 0·03% of the racemate) was observed from the oxidation of (±)-reticuline with potassium ferricyanide in a two-phase, chloroform–aqueous sodium hydrogen carbonate, system. Manganese dioxide, potassium nitrosodisulphonate, potassium ferricyanide in homogeneous solution, and ferric chloride all gave lower yields (see Experimental section). A stereochemical correlation between (−)-reticuline (VIII; R = H) and (+)-salutaridine (I) and hence thebaine (III) was achieved as follows. (+)- and (−)-Reticuline were separately labelled with tritium as described above. Each was oxidised, with ferricyanide in aqueous sodium hydrogen carbonate, under the same conditions, and the resulting oxidation products diluted with (+)-salutaridine. The diluted material from the oxidation of (−)-reticuline retained the activity on purification and conversion into thebaine (radiochemical yield 0·0044%); that from the oxidation of (+)-reticuline lost essentially all its activity on recrystallisation. A parallel oxidation of (±)-reticuline gave (+)-salutaridine in 0·0021% yield.

This particular oxidising system, though relatively inefficient, was chosen since the reaction conditions were reproducible and the product easy to purify. The very low yields of salutaridine observed from the oxidation of reticuline demand some explanation. Even if no intermolecular coupling took place oxidative cyclisation could give several products: aporphines [10,11] [8–6′ and 8–2′ coupling, see (VIII)], an isomer of salutaridine (4a–6′), and salutaridine itself (4a–2′). More importantly, we have found that salutaridine is oxidised more rapidly than reticuline and might therefore be largely destroyed under the conditions of its formation. Thus when a mixture of (+)-salutaridine (1 mole) and (±)-reticuline (1 mole) was treated with potassium ferricyanide (2·1 mole) the reaction products contained no salutaridine, but substantial amounts of reticuline were recovered. A new crystalline compound, presumably 1,1′-dehydrodisalutaridine, was isolated from this experiment.

Finally, (+)-salutaridine has been correlated with (−)-laudanosine (VIII; R = Me). Methylation of (+)-salutaridine, with methyl toluene-*p*-sulphonate and sodium hydride in dimethylformamide, gave the corresponding *O*-methyl ether. Cava *et al.*[12] have shown that dienones of the proaporphine groups are readily cleaved by sodium in liquid ammonia to the related benzylisoquinolines. However, similar reduction of *O*-methylsalutaridine gave a complex mixture of products. Again, radiochemical methods proved valuable in the preliminary investigation of the reduction mixture. *O*-Methyl-[1-^{3}H]salutaridine was reduced with sodium in liquid ammonia and the phenolic products methylated with diazomethane. Repeated column and thin-layer chromatography gave a small amount of material inseparable from (±)-laudanosine on a thin-layer chromatogram. The rotation, $[\alpha]_D$ −79° (in EtOH) and −54° (in $CHCl_3$), corresponded to that of (−)-laudanosine,[13] [α] −90° (in EtOH) and −53° (in $CHCl_3$), but insufficient material was available for adequate conventional characterisation. The product was therefore diluted with a large excess of (±)-laudanosine. The specific activity of the mixture remained constant on repeated recrystallisation, showing that the product was indeed laudanosine. Further, the specific activity of the reduction product, calculated from that of the crystalline diluted material, agreed well with the specific activity of the original *O*-methyl-[^{3}H]salutaridine. Thus the product must have been substantially pure (−)-laudanosine. The reductive cleavage was then carried out on a larger scale with inactive (+)-*O*-methylsalutaridine to give eventually crystalline (−)-laudanosine, $[\alpha]_D$ −86° (in EtOH), m. p. 88—89° undepressed on admixture with authentic material.

EXPERIMENTAL

Melting points were taken on a Kofler hot-stage apparatus. N.m.r. spectra were run on a Varian A-60 spectrometer on permanent loan (to D. H. R. B.) from the Wellcome Trust. Mass spectra were measured with an A.E.I. M.S.9, double focusing, spectrometer with an ionising potential of 70 ev. Samples were directly inserted on a probe. We thank Dr. E. S. Waight and his colleagues for these measurements.

Calcium D-*Glycerate*.— 1,2,5,6-Di-isopropylidene-D-mannitol [14] (4 g.) in water (1 l.) containing potassium carbonate (6 g.) was oxidised [15] with potassium periodate (16 g.) and potassium permanganate (0·34 g.). The reaction mixture was warmed and more water (750 ml.) added to dissolve most of the periodate. The mixture was stirred at room temperature for 18 hr. and then warmed to precipitate manganese dioxide. This was filtered off and the filtrate evaporated *in vacuo* to dryness. The residue was washed with ether (3 × 50 ml.) and extracted with ethanol (5 × 50 ml.). The extract was concentrated to 15 ml., then diluted with ether to give crystalline potassium isopropylidene-D-glycerate [16] (2·1 g.). Recrystallisation from ethanol–ether gave hygroscopic material (dried *in vacuo* at 130°), m. p. 235—236°, $[\alpha]_D$ +41° (*c* 0·59 in H_2O). Treatment with brucine hydrochloride in ethanol gave the corresponding brucine salt which, after crystallisation from ethanol–ether, had m. p. 254—258° softening at 90—100°. Potassium isopropylidene-D-glycerate, m. p. 232—234°, [α] +35° (*c* 2 in H_2O), was regenerated from this brucine salt by treatment with aqueous potassium carbonate.

Potassium isopropylidene-D-glycerate (12 g.) was heated at *ca.* 50° in 10% aqueous acetic acid (50 ml.) for 30 min.

[11] B. Franck and G. Schlingloff, *Annalen*, 1962, **659**, 123; S. M. Albonico, A. M. Kuck, and V. Deulofeu, *ibid.*, 1965, **685**, 200; A. H. Jackson and J. A. Martin, *Chem. Comm.*, 1965, 420.
[12] M. P. Cava, K. Momura, R. H. Schlessinger, K. T. Buck, B. Douglas, R. F. Raffauf, and J. A. Weisbach, *Chem. and Ind.*, 1964, 282.
[13] A. Burger in "The Alkaloids," ed. R. H. F. Manske and H. L. Holmes, Academic Press, New York, 1954, vol. IV, p. 56.
[14] E. Baer, *J. Amer. Chem. Soc.*, 1945, **67**, 338.
[15] R. V. Lemieux and E. von Rudloff, *Canad. J. Chem.*, 1955, **33**, 1701.
[16] Cf. T. Reichstein, A. Pedolin, and A. Grüssner, *Helv. Chim. Acta*, 1935, **18**, 598.

Ion-exchange resin (Amberlite I.R. 120, H^+ form) was added to remove potassium ions and the suspension filtered. The filtrate was evaporated *in vacuo* and the oily residue dissolved in water and treated with excess of calcium carbonate. The mixture was heated for 2 hr. and then filtered while hot. Concentration and refrigeration of the filtrate gave crystalline calcium D-glycerate dihydrate (3·0 g.), $[\alpha]_D$ +13·6° (*c* 4·4 in H_2O) [lit.,[4] $[\alpha]_D$ +14·4° (*c* 2·12 in H_2O)].

Calcium L-*Glycerate*.—L-Serine was deaminated with nitrous fumes in the usual way.[17] The crude calcium L-glycerate was purified *via* *p*-bromophenacyl L-glycerate:[4] chromatography on silica gel (Hopkin and Williams Ltd., M.F.C.) gave material, m. p. 114—115°, $[\alpha]_D$ +1·5° (*c* 1·80 in acetone) [lit.[4], m. p. 109—111°, $[\alpha]_D$ −1·9° (*c* 5·62 in acetone), for the enantiomer]. This ester was hydrolysed with hot aqueous-ethanolic calcium hydroxide to give pure calcium L-glycerate dihydrate, $[\alpha]_D$ −12·3° (*c* 1·7 in H_2O) [lit.,[17] $[\alpha]_D$ −14·6° (in H_2O)].

Ozonolysis of the Salutaridinols.—[7-^{3}H]Salutaridinol-I (28 mg., 1625 counts/sec./mg.) was ozonised in ethanol (50 ml.) at −70° for 30 min. The solution was flushed with oxygen to remove excess of ozone and allowed to warm to room temperature. Adams catalyst (25 mg.) and a trace of hydrochloric acid were added and the mixture hydrogenated overnight. The solution was filtered and adjusted to *ca.* pH 10 with potassium hydroxide. After 24 hr. at room temperature, non-radioactive calcium D-glycerate dihydrate (30 mg.) was added and the solution adjusted to pH 6 with hydrochloric acid. *p*-Bromophenacyl bromide (500 mg.) was added and the solution heated under reflux overnight. Most of the solvent was evaporated and the residue diluted with water (20 ml.) and extracted with methylene dichloride (3 × 30 ml.). The extract was dried ($MgSO_4$) and evaporated, and the residue chromatographed on silica gel (Hopkin and Williams Ltd. M.F.C.). Elution with ethyl acetate–benzene (1 : 1) gave *p*-bromophenacyl D-glycerate (18 mg.). This was diluted with non-radioactive ester (14 mg.) and the mixture recrystallised from benzene to a constant activity of 3·2 counts/sec./mg., corresponding to a 0·80% yield of D-glyceric acid from salutaridinol-I. Similar degradations were carried out on salutaridinol-II using D-glyceric acid for dilution, and on both salutaridinols using L-glyceric acid for dilution. The results are tabulated (see Theoretical section).

Tritiation of Reticuline.—Tritiated hydrochloric acid was generated from tritiated water (0·3 ml.) and thionyl chloride (48 mg.). (±)-Reticuline (100 mg.) was heated in this solution for 96 hr. at 100° then recovered by dilution with excess of N-sodium hydrogen carbonate and extraction with chloroform. The extract was evaporated and the residue chromatographed on grade V alumina (20 g.), elution with chloroform–ethanol (100 : 4) giving (±)-[^{3}H]-reticuline (90 mg.). An experiment using deuterium oxide showed (n.m.r. control) that exchange of all aryl protons, and no others, occurred. Similarly (+)- and (−)-reticuline were tritiated to give the corresponding hydrochlorides, $[\alpha]_D$ +72° and −72° (*c* 0·5 in H_2O), respectively [lit.,[9] $[\alpha]_D$ +73° and −75° (*c* 1·0 in H_2O)].

Oxidation of (±)-*Reticuline* (with Dr. G. M. Thomas).—To a mixture of tritiated (±)-reticuline (52 mg.) in chloroform (50 ml.), and sodium hydrogen carbonate (200 mg.) in water (50 ml.), under nitrogen at room temperature, was added potassium ferricyanide (110 mg.) in water (50 ml.) during 30 min. with stirring. After 1 hr. non-radioactive (+)-salutaridine (50 mg.) was added. The chloroform layer was removed and the aqueous layer extracted with chloroform (15 × 3 ml.). The combined chloroform solutions were washed with water, dried (Na_2SO_4), and evaporated. The residue was chromatographed on grade III alumina (25 g.), elution with chloroform–benzene (1 : 1) giving salutaridine (45 mg.) which was crystallised to constant activity. The activity was used to calculate the yield (0·015%) of (+)-salutaridine formed in the oxidation, allowance being made for the loss of one tritium atom during oxidative cyclisation. The radioactive (+)-salutaridine was converted[3] *via* the salutaridinols into thebaine, which had the same molar activity.

Similarly, the yields of (+)-salutaridine were determined from the oxidation of tritiated (±)-reticuline under the following conditions (per cent yields in parenthesis): (±)-reticuline (68 mg.) in chloroform (5 ml.) treated with manganese dioxide (236 mg.) for 3 hr. at room temperatures (0·011); (±)-reticuline (28 mg.) and (+)-salutaridine (40 mg.) in chloroform (50 ml.), and sodium acetate (70 mg.) in water (50 ml.), treated with potassium nitrosodisulphonate (53 mg.) in water (50 ml.) for 1 hr. at 0° (0·0054); (±)-reticuline (54 mg.) oxidised with ferric chloride (130 mg.) in water (150 ml.) at room temperature for 20 hr. (*ca.* 0·0007). All oxidations, except that with manganese dioxide, were carried out under nitrogen.

Oxidation of Optically Active Reticuline.—Potassium ferricyanide (114 mg.) in water (50 ml.) was added dropwise, with stirring, during 45 min. to tritiated (−)-reticuline (10 mg.) and unlabelled (±)-reticuline (43 mg.) in water (50 ml.) and N-sodium hydrogen carbonate (25 ml.), under nitrogen at 0°. After 2 hr., inactive (+)-salutaridine (90 mg.) was added, and then recovered and purified in the usual way. The molar activity did not drop upon conversion into thebaine. The yield of (+)-salutaridine from (−)-reticuline was 0·0044%. Similar experiments with tritiated (±)-reticuline and (+)-reticuline gave yields of 0·0021 and <0·0003%, respectively.

Oxidation of Salutaridine and Reticuline.—Potassium ferricyanide (114 mg.) in water (50 ml.) was added during 30 min. with stirring to (+)-salutaridine (50 mg.) and (±)-reticuline (55 mg.) in water (80 ml.) and N-sodium hydrogen carbonate (10 ml.) at 0° under nitrogen. After 1 hr. the product was extracted into chloroform (15 × 5 ml.) and chromatographed on grade III alumina (30 g.) in the usual way. Elution with benzene–chloroform (1 : 1) gave no salutaridine. Elution with chloroform gave a compound (30 mg.) which crystallised from chloroform–ether as needles, m. p. 242—245°, $\nu_{max.}$ 3560, 1670, 1642, and 1624 cm.$^{-1}$ (in $CHCl_3$). The i.r. spectrum resembled that of salutaridine but lacked the sharp band at 1494 cm.$^{-1}$ characteristic[3] of an unsubstituted 1-position. This substance was presumably 1,1′-dehydrodisalutaridine but was not investigated further. Elution with chloroform–ethanol (100 : 4) gave (±)-reticuline (40 mg.).

O-*Methylsalutaridine*.—(+)-Salutaridine (1 g.) in dimethylformamide (10 ml.) was treated, under nitrogen, with sodium hydride (55% dispersion in mineral oil, 160 mg.) also in dimethylformamide (5 ml.). After the evolution of hydrogen had ceased (*ca.* 30 min.), methyl toluene-*p*-sulphonate (500 mg.) in dimethylformamide (5 ml.) was added and the mixture stirred at room temperature for 4 hr. The solvent was evaporated *in vacuo* and

[17] E. Fischer and W. A. Jacobs, *Ber.*, 1907, **40**, 1068.

the residue treated with water (10 ml.) containing 4N-sodium hydroxide (*ca.* 0·5 ml.). Extraction with ethyl acetate (3 × 10 ml.) gave the desired non-phenolic product. This crude ether was chromatographed twice on silica gel (British Drug Houses Ltd.) (100 g.), elution with ethyl acetate and ethyl acetate–methanol (1 : 1) giving substantially pure material (420 mg.) contaminated with a yellow substance. Crystallisation from ethanol–ether gave *O*-methylsalutaridine as prisms (300 mg.), m. p. 147—148°. The molecular formula was determined by mass spectroscopy (Found: *M*, 341·16269. $C_{20}H_{23}NO_4$ requires *M*, 341·16475). The n.m.r. spectrum in $CDCl_3$ showed the following signals (τ values): *N*-Me (7·51), *O*-Me (6·20, 6·14, 6·06), H_8 (3·67), H_1 and H_2 (3·14), and H_5 (2·70).

Reductive Cleavage of O-*Methyl*-[1-^{3}H]-(+)-*salutaridine*.—*O*-Methyl-[1-^{3}H]-(+)-salutaridine [3] (420 mg., 7·1 × 10^{-2} mc/mmole) in tetrahydrofuran (20 ml.) and liquid ammonia (250 ml.) (distilled from sodium) was treated with sodium at −20° until a permanent blue colour was obtained. After 30 min. ethanol was added to give a clear solution which was allowed to evaporate to 50 ml. at room temperature. This solution was poured into water (500 ml.) and excess of solid carbon dioxide added. The products were extracted into ethyl acetate. Evaporation gave an oil which was redissolved in ethyl acetate and separated into phenolic and non-phenolic fractions with aqueous sodium hydroxide in the usual way. The phenolic fraction (55 mg.), in methanol, was methylated with excess of ethereal diazomethane for 2 days to give an oil (48 mg.). The " non-phenolic " fraction (110 mg.) was treated in the same way to give an oil (90 mg.). Thin-layer chromatography (t.l.c.) on silica gel G (Merck) plates, developed with methanol, showed that both methylated fractions contained at least ten components. Both contained one component running alongside laudanosine and showing in ultraviolet light a characteristic blue fluorescence after brief exposure to iodine vapour. The two fractions were chromatographed separately on grade III alumina, elution with benzene then benzene–ethyl acetate (9 : 1) giving fractions enriched in laudanosine. Further purification by t.l.c. on silica gel gave a fraction (1·5 mg.) showing only one component inseparable from (±)-laudanosine. This material had $[\alpha]_D$ −79° (*c* 0·13 in EtOH) and −54° (*c* 0·088 in $CHCl_3$) corresponding to (−)-laudanosine [lit.,[13] $[\alpha]_D$ +90° (*c* 1·42 in EtOH) and +52° (*c* 1·67 in $CHCl_3$) for the enantiomer]. The (−)-laudanosine was recovered from the rotation measurement and its weight (0·66 mg.) determined by comparison of the u.v. spectrum with that of pure (±)-laudanosine. This was diluted with non-radioactive (±)-laudanosine (22·4 mg.) and the mixture crystallised to constant specific activity (2·2 × 10^{-3} mc/mmole), corresponding to an activity before dilution of 7·8 × 10^{-2} mc/mmole. This value agrees reasonably with that (7·1 × 10^{-2} mc/mmole) of the original *O*-methylsalutaridine, showing that the (−)-laudanosine was substantially pure.

(−)-*Laudanosine from* O-*Methyl*-(+)-*salutaridine*.—*O*-Methyl-(+)-salutaridine (1·05 g.) was cleaved with sodium in liquid ammonia (as above). The reaction products were methylated with diazomethane without preliminary separation into phenolic and non-phenolic fractions. Repeated column and t.l.c. on alumina and silica gel gave (−)-laudanosine (8 mg.) as an oil. Seeding of a light petroleum (b. p. 60—80°) solution with authentic (−)-laudanosine [18] and recrystallisation from the same solvent gave needles (1·5 mg.), m. p. and mixed m. p. 88—89°, $[\alpha]_D$ −86° (*c* 0·072 in EtOH).

Financial support from the S.R.C. and from the Wellcome Trust is gratefully acknowledged.

[6/1061 *Received, August 22nd,* 1966]

[18] G. K. Hughes, E. Ritchie, and W. C. Taylor, *Austral. J. Chem.*, 1953, **6**, 315.