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The Metabolic Interconversion of Arecoline and Arecoline 1-Oxide in the Rat

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1. Tritiation of arecoline hydrochloride by catalytic exchange in aqueous media (done by The Radiochemical Centre) gave arecaidine hydrochloride of high specific radioactivity; this on treatment with diazomethane gave [^{3}H]arecoline, which was oxidized with peroxyacetic acid to [^{3}H]arecoline 1-oxide. 2. Arecoline 1-oxide gave arecaidine 1-oxide on acid hydrolysis and 1,2-dihydro-1-methylnicotinic acid methyl ester on thermal decomposition. 3. [^{3}H]Arecoline hydrochloride was metabolized in the rat into the ^{3}H-labelled derivatives of arecoline 1-oxide, arecaidine 1-oxide, arecaidine, *N*-acetyl-*S*-(3-carboxy-1-methylpiperid-4-yl)-L-cysteine and an unidentified metabolite; some unchanged arecoline was also excreted. [^{3}H]Arecoline 1-oxide gave the same metabolities, but in different amounts. 4. The possible relevance of these findings to betel-nut carcinogenesis is discussed.

The chewing of betel-nut preparations is a widespread Oriental habit which often spans the lifetime of the chewer and produces euphoria, addiction, dizziness, sweating, excessive salivation and a sensation of tightness in the throat. Various concoctions of the nut or of other parts of the areca palm are, or have been reputedly, used as treatment for diarrhoea, intestinal worms and other gastro-intestinal disorders, gonorrhoea, ailments of vision, fever, dysentery, hysteria, schizophrenia, headaches, dental disorders, octopus bites and elephant diseases and as an abortifacient (Burkhill, 1935; Goswami & Ahmed, 1956; Arai & Nakayama, 1952). There is some dispute as to whether this apparent panacea is associated with submucous fibrosis of the buccal cavity (For: Paymaster, 1962; Pindborg, 1965; Pindborg, Poulsen & Zachariah, 1967. Against: Khanolkar, 1944; Balendra, 1949; Tennekoon & Bartlett, 1969), but it is generally agreed that there is a parallelism between the prevalence of the betel-chewing habit and the incidence of cancers of the oropharynx (Muir & Kirk, 1960; Muir, 1967; Schonland & Bradshaw, 1969). The composition of the quid shows regional and local variations, but the essential ingredients are the dried areca nut (*Areca catechu* L.) and lime with or without tobacco; the mixture is wrapped in the mature leaves of the betel vine (*Piper betel*). The cancers may be due to the combined action of the ingredients of the quid, or of the nuts and lime (Schonland & Bradshaw, 1969) or of the alkaloids of the nut (Boyland, 1968). The active principle of the nut is arecoline (1,2,5,6-tetrahydro-1-methylnicotinic acid methyl ester; I, Scheme 1) which may comprise up to 0.8% by weight of the ripe nut (Goswami & Ahmed, 1956). Arecoline is a cathartic and vermifuge which exerts cholinergic, sialogogic, diaphoretic, parasympathomimetic and other effects similar to those produced by pilocarpine, muscarine and nicotine; it is probably the active principle, since the other betel-nut alkaloids do not show these effects or do so to a lesser extent (Burkhill, 1935; von Euler & Domeij, 1945; Goswami & Ahmed, 1956; Muir & Kirk, 1960; Pfeiffer, Beck & Goldstein, 1967; Nieschulz & Schmersahl, 1968; Boyland & Nery, 1969). This work describes the metabolic interconversion of arecoline and arecoline 1-oxide in the rat.

MATERIALS AND METHODS

Measurement of radioactivity. This was done with a Packard Tri-Carb model 3375 liquid-scintillation spectrometer and values represent counts at least 50% above background, corrected for quenching by the addition of an internal standard. Paper strips cut off chromatograms or silica gel scraped off t.l.c. plates were assayed for radioactivity in glass phials containing 10ml of scintillation fluid consisting of a solution of naphthalene (80g), 2,5-diphenyloxazole (5g) and 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (0.05g) in a mixture of toluene (385ml), dioxan (385ml) and methanol (230ml). Nucleic acids and proteins isolated from livers and acetone-dried powders were assayed as follows. DNA (2–5mg) was hydrolysed for 16h at 37°C in aq. 0.1% (w/v) deoxy-

ribonuclease I solution (0.5 ml; 75000 units), and RNA similarly in aq. 0.1% (w/v) ribonuclease solution (0.5 ml; 50000 units) and proteins and acetone-dried powders (2–5 mg) were dissolved in tetraethylammonium hydroxide solution (1.0 ml); scintillation fluid (10 ml) was added to each mixture and the radioactivity determined.

Metabolic experiments. [^{3}H]Arecoline hydrochloride (sp. radioactivity, 1.72 Ci/mol) was administered by gavage to 6-week-old female Chester Beatty rats as an aq. 1% (w/v) solution (dose, 50 mg/kg body wt.). The animals, which were starved for 8 h before and for the duration of the experiments, were housed in polythene cages lined with two sheets of Whatman 3MM chromatography paper held in position with glass rods. Water was available *ad lib.*

Preparation of acetone-dried powders of tissues and isolation of liver nucleic acids and proteins. Experimental animals were killed by cervical dislocation and freshly excised tissues were homogenized in 10 vol. of acetone in an Ultra-Turrax homogenizer with a Teflon pestle. The homogenate was filtered and the residue washed with acetone until the final washing contained no radioactivity. DNA, RNA and proteins were isolated from freshly excised livers by the method of Kirby (1956) as modified by Brookes & Lawley (1965), but the original method was used to remove polysaccharides. DNA was deproteinized by the detergent–salt procedure of Kay, Simmons & Dounce (1952); RNA was further purified by the Kirby (1956) phenol method and protein by repeated precipitations with acetone from solution in aq. 5% (w/v) NaCl until the final supernatant contained no residual radioactivity after evaporation.

Chromatography. Whatman no. 1 chromatography paper (unless otherwise stated) was used for overnight downward development in the following solvent systems: (*a*) butan-1-ol–propan-1-ol–aq. 2M-NH_3 (2:1:1, by vol.) and (*b*) butan-1-ol–acetic acid–water (12:3:5, by vol.); and for ascending chromatography in (*c*) methanol–6M-HCl (7:3, v/v). For t.l.c., glass plates were coated with films (0.25 mm thick) of silica gel G (E. Merck A.-G., Darmstadt, Germany) and the chromatograms were developed in (*d*) ethanol–light petroleum (b.p. 40–60°C)–2M-NH_3 (6:1:1, by vol.) and (*e*) ethanol–acetic acid–water (1:1:8, by vol.). For the detection of compounds on chromatograms, other than by radioactivity assays, the following reagents were used: (i) ammoniacal aq. 2% (w/v) $AgNO_3$; (ii) 0.1M-$K_2Cr_2O_7$–acetic acid (1:1, v/v) followed by 0.1M-$AgNO_3$; (iii) aq. 5% (w/v) $KBiI_4$–10M-HCl (200:1, v/v) (Bartley, 1954); (iv) sodium amminoprusside (Boyland & Nery, 1965) and (v) 0.2M-NH_2OH [made by adding a solution of NH_2OH,HCl (1.38 g) in methanol (50 ml) to a similar solution of KOH (1.1 g) at 0°C immediately before use] followed by heating the chromatograms at 60°C for 10 min and spraying with ethanolic 1% (w/v) $FeCl_3$–2M-HCl (99:1, v/v).

N-Acetyl-*S*-(3-carboxy-1-methylpiperid-4-yl)-L-cysteine (V, Scheme 1) gave a brown spot with reagent (ii); 1-methylpiperidines (I–VII) gave faint pink to red spots with reagent (iii); arecoline (I) and arecaidine (II) reduced reagent (i) after 3–4 h; methyl esters (I, III, VI and VII) gave red to purple spots with reagent (v); arecoline *N*-oxide (III) and 3,4-epoxy-1,2,5,6-tetrahydro-1-methylnicotinic acid methyl ester 1-oxide (VII) gave purple spots with reagent (iv).

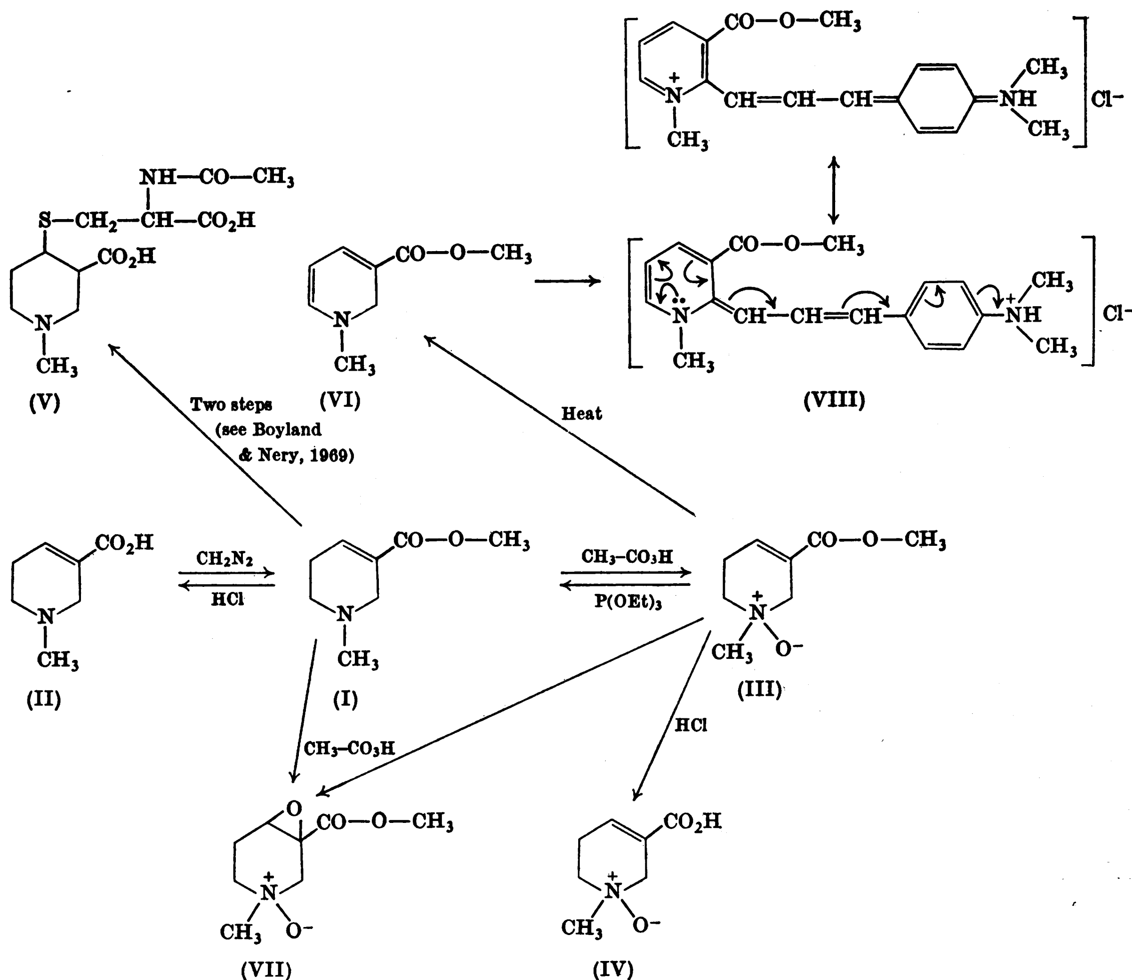
Chemicals. Arecoline and ribonuclease A were purchased from Sigma Chemical Co., St Louis, Mo., U.S.A.; *N*-acetyl-L-cysteine was purchased from British Drug Houses Ltd., Poole, Dorset, U.K., and deoxyribonuclease I (D) from Worthington Biochemical Corp., Freehold, N.J., U.S.A. Arecaidine and arecaidine hydrochloride were prepared as described by Boyland & Nery (1969).

[^{3}H]1,2,5,6-*Tetrahydro*-1-*methylnicotinic acid* (*arecaidine*, II, *Scheme* 1). Arecoline hydrochloride (2 g) was tritiated by catalytic exchange in aqueous media and the mixture freed from catalyst. This was performed by The Radiochemical Centre, Amersham, Bucks., U.K. The sample as received contained 4.5 Ci and examination by chromatography in solvent system (*d*) showed that it contained mainly arecaidine, i.e. the ester had been converted into the free acid either by catalytic reduction or by hydrolysis. The solution was neutralized with solid Na_2CO_3, evaporated *in vacuo* and the residue extracted with hot methanol (3 × 30 ml). The extracts were combined and acidified to pH 2 with 10M-HCl, evaporated *in vacuo* and the residue was recrystallized from aq. 80% (v/v) ethanol to yield colourless plates of [^{3}H]arecaidine hydrochloride (1.2 g; sp. radioactivity, 301 Ci/mol; 2.04 Ci or 46% of radioactivity received), m.p. and mixed m.p. with the unlabelled compound 265°C (decomp). Chromatography in solvents (*a*), (*b*), (*d*) and (*e*) showed that the compound was indistinguishable from arecaidine hydrochloride.

[^{3}H]1,2,5,6-*Tetrahydro*-1-*methylnicotinic acid methyl ester* (*arecoline*, I, *Scheme* 1). A solution of [^{3}H]arecaidine hydrochloride (0.1 g, 170 mCi) in methanol (2 ml) was treated with diazomethane (approx. 2 g) in ether (150 ml) in portions (15 ml) during 3 h. After 18 h the mixture was evaporated, the residue dissolved in a solution of arecoline hydrochloride (5.0 g) in 1M-HCl (10 ml) and the solution evaporated to dryness *in vacuo*. The residue was recrystallized from propan-1-ol to constant radioactivity to yield colourless needles (4.6 g; sp. radioactivity 1.72 Ci/mol) of [^{3}H]arecoline hydrochloride, m.p. and mixed m.p. with the unlabelled compound, 158°C. Chromatography in solvents (*a*), (*b*), (*d*) and (*e*) showed that the compound was indistinguishable from arecoline hydrochloride.

1,2,5,6-*Tetrahydro*-1-*methylnicotinic acid methyl ester* 1-*oxide* (*arecoline* 1-*oxide*, III, *Scheme* 1). A solution of arecoline (12.2 g, 0.08 mol) in ether (50 ml) was stirred at 0°C and treated with aq. 33% (w/v) peroxyacetic acid (22.8 g, approx. 0.1 mol) dropwise during 30 min. After 2 h the oily lower layer which separated was dissolved in ethanol and precipitated three times by the addition of ether and dried at 65°C/15 mmHg over P_2O_5 to give arecoline 1-oxide as a pale yellow viscous oil (10.6 g, yield 79%) (Found: C, 56.5; H, 7.6; N, 7.8; $C_8H_{13}NO_3$ requires C, 56.1; H, 7.65; N, 8.2%). Chromatography in solvents (*a*), (*b*) and (*d*) showed only one component. The compound was soluble in acetone, ethanol and water and insoluble or slightly soluble in light petroleum (b.p. 60–80°C), ether and chloroform. A sample (20 mg) of the compound in chloroform (10 ml) containing triethyl phosphite (0.2 ml) was heated under reflux for 30 min; chromatography of a sample of the reaction mixture in solvents (*a*) and (*b*) revealed that the compound was converted into arecoline.

[^{3}H]1,2,5,6-*Tetrahydro*-1-*methylnicotinic acid methyl*



Scheme 1. Summary of the reactions of arecoline and arecoline 1-oxide described in the text. Arrows indicate chemical reactions. Compounds (I) to (V) were excreted in the urine after administration of arecoline or arecoline 1-oxide to rats. Other details are given in the text.

ester 1-oxide. A solution of [^{3}H]arecoline hydrochloride (1.9g, 17.2mCi) in 10M-NaOH (1ml) at 0°C was extracted with chloroform (3×5ml); the extracts were combined, dried (over Na_2SO_4) and evaporated; the residue was dissolved in ether (10ml) and treated with 33% peroxyacetic acid (4ml). As described for the unlabelled analogue, chromatographically homogeneous [^{3}H]arecoline 1-oxide (0.85g; sp. radioactivity, 1.72Ci/mol) was obtained.

1,2-Dihydro-1-methylnicotinic acid methyl ester (VI, *Scheme* 1). Arecoline 1-oxide (6g) was heated at 140°C for 1h. The product was cooled and extracted with hot light petroleum (b.p. 60–80°C) (3×20ml). The combined extracts were washed with water (2×10ml), dried (over Na_2SO_4) and evaporated *in vacuo*, and the residue was recrystallized from light petroleum (b.p. 60–80°C) to give lustrous colourless plates of 1,2-dihydro-1-methylnicotinic acid methyl ester (0.4g), m.p. 91°C (Found: C, 62.4; H, 7.4; N, 9.2%; mol.wt. 151. $C_8H_{11}NO_2$ requires C, 62.7; H, 7.2; N, 9.1%; mol.wt. 153). This compound was also obtained as a colourless oil, b.p. 88–92°C/0.05mmHg, during an attempt to distil arecoline 1-oxide (10g) when the bath temperature reached 160°C. The oil solidified on cooling and the solid was recrystallized as before to give lustrous colourless plates (0.6g) of compound (VI), m.p. and mixed m.p. 91°C. The compound had a mousy odour, was insoluble in water and soluble in ether, acetone, ethyl acetate and ethanol.

1,2-Dihydro-2(4′-dimethylaminocinnamylidenyl)-1-methylnicotinic acid methyl ester chloride (VIII, *Scheme* 1). A solution of 1,2-dihydro-1-methylnicotinic acid methyl ester (0.15g) and 4-dimethylaminocinnamaldehyde (0.2g) in ethanol (10ml) containing 10M-HCl (0.4ml) was heated under reflux for 10min, the resulting blue solution evaporated *in vacuo* and the residue recrystallized from

propan-1-ol to give blue prisms, which decomposed with partial melting on being heated above 250°C, of *1,2-dihydro-2(4′-dimethylaminocinnamylidenyl)-1-methylnicotinic acid methyl ester chloride* (0.25g, yield 72%) (Found: C, 65.9; H, 6.6; Cl, 9.6; N, 7.7; $C_{19}H_{23}ClN_2O_2$ requires C, 65.8; H, 6.7; Cl, 10.2; N, 8.1%). It showed $\lambda_{max.}$ 620nm and 642nm in 0.1M-HCl and 0.1M-$NaHCO_3$ respectively.

1,2,5,6-Tetrahydro-3,4-epoxy-1-methylnicotinic acid methyl ester 1-*oxide* (VII, *Scheme* 1). A solution of arecoline (1.55g, 0.01mol) in acetone (20ml) was treated with 33% peroxyacetic acid (11.4g, approx. 0.05mol) and the resulting solution evaporated *in vacuo* after 16h at 23°C. The residual oil was dissolved in ethanol (5ml) and precipitated three times by addition of ether to give a colourless gum (1.2g), which failed to solidify by normal techniques. It gave a reineckate [pink plates, m.p. 122°C (decomp.)], after recrystallization from water. The reineckate corresponded to the structure: $(C_8H_{14}NO_4)^+$ $[Cr(NH_3)_2(SCN)_4]^-$ $C_8H_{13}NO_4,H_2O$ (Found: C, 33.8; H, 4.6; N, 16.2; S, 18.6; weight loss by drying at 62°C/15mmHg over P_2O_5 for 16h, 2.4%. $C_{20}H_{35}CrN_8O_9S_4$ requires C, 33.7; H, 5.0; N, 15.7; S, 18.0; H_2O, 2.5%.) Similar results were obtained from the oxidation of arecoline (1.55g) with aq. 30% (v/v) hydrogen peroxide (5ml) at 23°C for 16h.

1,2,5,6-Tetrahydro-1-methylnicotinic acid 1-*oxide* (*arecaidine* 1-*oxide*, IV, *Scheme* 1). A solution of arecoline 1-oxide (1.7g) in 10M-HCl (10ml) was heated at 100°C for 3h, evaporated *in vacuo* and the residue recrystallized from propan-1-ol to yield colourless prisms of *arecaidine* 1-*oxide chloride* (1.2g) m.p. 168°C (decomp.). (Found: C, 43.7; H, 6.3; Cl, 18.1; N, 7.1; $C_7H_{12}ClNO_3$ requires C, 43.6; H, 6.2; Cl, 18.2; N, 7.25%). A solution of the chloride (0.2g) in aq. 50% (v/v) methanol (10ml) containing picric acid (0.23g) after 15min heating at 60°C was evaporated *in vacuo* and the residue recrystallized from water to give orange needles of *arecaidine* 1-*oxide picrate* (0.36g), m.p. 198°C (decomp.) (Found: C, 40.1; H, 3.6; N, 14.5. $C_{13}H_{14}N_4O_{10}$ requires C, 40.4; H, 3.65; N, 14.5%). Oxidation of arecaidine (0.1g) in water (1ml) with 30% hydrogen peroxide (0.5ml) or with 33% peroxyacetic acid (0.5ml) at 23°C and examination of the mixtures by chromatography in solvent (*d*) at hourly intervals during 8h revealed that the oxidation of arecaidine proceeded slowly and detectable amounts of arecaidine 1-oxide were formed after 4h.

RESULTS

Metabolism of [^{3}H]*arecoline*. [^{3}H]Arecoline hydrochloride (total dose 40mg, 360μCi) was administered to four rats and the urine and faeces were collected during 18h. Extraction of the faeces with aq. 80% methanol (2×30ml) followed by measurement of radioactivity of the residue obtained after evaporation of the combined extracts revealed that approx. 1.2% of the administered radioactivity was excreted in the faeces; this was not further examined. The glass rods, filter paper and inside of the cage were extracted with aq. 80% methanol in 100ml portions until the final extract contained negligible radioactivity as determined by scintillation counting. The extracts were combined and evaporated to dryness *in vacuo*, and the residue was dissolved in water (15ml). Measurement of radioactivity of samples (0.1ml) showed that approx. 19.5% of the administered radioactivity was excreted in the urine. The rate of excretion of the remaining radioactivity was not investigated. Samples (0.1 ml) were chromatographed in solvents (*a*) and (*b*) on Whatman 3MM chromatography paper and strips (2cm wide and 1cm along the direction of development) were cut off and assayed for radioactivity. This showed the presence of six radioactive peak areas, corresponding to the metabolites shown in Table 1. The amounts of the metabolites were determined from the radioactivity of each peak area and are expressed as a percentage of the total radioactivity (method *A*). Metabolites I to V were also determined by isotope-dilution analysis (method *B*), as follows: a sample (0.5g) of arecoline hydrochloride, arecaidine hydrochloride, arecaidine 1-oxide chloride or *N*-acetyl-*S*-(3-carboxy-1-methylpiperid-4-yl)-L-cysteine was dissolved in the minimum volume of 0.1M-hydrochloric acid and treated with the urine concentrate (0.5ml). The mixtures were evaporated to dryness *in vacuo* and the residues recrystallized to constant radioactivity from propan-1-ol, aq. 80% ethanol, propan-1-ol and ethanol respectively. The chemical purity of each constant-radioactivity product so obtained was checked by mixed-m.p. determinations and by chromatography in solvents (*a*) and (*b*); the radiochemical purity was checked by chromatography in solvent (*a*) followed by determination of radioactivity of the appropriate strip cut off the developed chromatogram. Arecoline 1-oxide (10mg) was dissolved in the urine concentrate (0.1ml); samples (0.01ml) were chromatographed in solvents (*d*) and (*e*) by the two-dimensional technique and the silica gel scraped off the appropriate region on the dried plate was assayed for radioactivity. The results obtained by methods *A* and *B* are shown in Table 1.

Binding of [^{3}H]*arecoline to rat tissues* in vivo. The rats used in the above experiments were killed after 18h and acetone-dried powders of various organs were prepared and assayed for radioactivity as described in the Materials and Methods section. The results, expressed as d.p.m./mg of dried powder and in decreasing order of binding capacity, were as follows: intestine, 1200; liver, 1050; lung, 770; stomach, 760; kidney, 500; bladder, 330; spleen, 230.

Binding of [^{3}H]*arecoline to proteins and nucleic acids of liver* in vivo. Four rats were killed 16h after administration of [^{3}H]arecoline hydrochloride (total dose 40mg, 360μCi); the freshly excised livers were fractionated into proteins and nucleic acids and assayed for radioactivity as described in the

Table 1. *Products excreted in the urine of rats after administration of* [^{3}H]*arecoline and* [^{3}H]*arecoline* N-*oxide*

Numbers in parentheses indicate percentage of radioactivity excreted after administration of the *N*-oxide. Roman numerals correspond to the structures shown in Scheme 1 and to those of compounds described in the text. For other details, see the text.

	Percentage of radioactivity excreted as determined by method		R_F of product in solvent system			
^{3}H-labelled product	*A*	*B*	*a*	*b*	*d*	*e*
I Arecoline	5.7	5.3 (1.8)	0.85	0.67	0.61	0.48
II Arecaidine	2.2	1.9 (1.4)	0.08	0.40	0.20	0.62
III Arecoline *N*-oxide	1.7	1.6 (3.6)	0.42	0.56	0.48	0.30
IV Arecaidine *N*-oxide	3.8	3.8 (5.2)	0.04	0.32	0.14	0.41
V *N*-Acetyl-*S*-(3-carboxy-1-methylpiperid-4-yl)-L-cysteine	1.4	1.3 (0.5)	0.01	0.33	0.28	—
VI Unknown	1.9	— —	0.22	0.11	—	—

Materials and Methods section. The bound radioactivity (d.p.m./mg of protein or nucleic acid) of each purified fraction was as follows: cytoplasmic protein, 84; nuclear protein, 110; DNA, 295; and RNA, 480. Hydrolysis of a sample (5mg) of the RNA in 1M-hydrochloric acid at 100°C for 1h and chromatography of the hydrolysate in solvents (*b*) and (*c*) showed that approx. 15% of the radioactivity associated with the RNA appeared at the origin and 80–85% between R_F0.9 and R_F1.0 in both chromatograms. No other area of significant radioactivity appeared on the chromatogram developed in solvent (*c*); an area of radioactivity centred at R_F0.56 and representing approx. 2% of the RNA radioactivity appeared on the other chromatogram. The macromolecules were not further investigated.

1-*Deoxygenation of arecoline* 1-*oxide* in vivo. [^{3}H]Arecoline 1-oxide (20mg, 180μCi) was administered to two rats. The urine was collected for 16h and a concentrate was prepared and analysed by method *B* for the presence of arecoline, arecaidine, arecoline 1-oxide, arecaidine 1-oxide and *N*-acetyl-*S*-(3-carboxy-1-methylpiperid-4-yl)-L-cysteine as described for the metabolism of [^{3}H]arecoline. The results (see Table 1) showed that arecoline 1-oxide was 1-deoxygenated *in vivo*.

DISCUSSION

The betel-nut alkaloids of known structures (arecoline, arecaidine, guvacine, guvacoline and choline), except choline, are derivatives of 1,2,5,6-tetrahydronicotinic acid; the others are isoguvacine (which may be impure arecaidine; Marion, 1950) and arecolidine (which may be 3,4-dimethoxy-1-methyl-1,2-dihydropyridine; Emde, 1915).

In common with many chemical carcinogens, arecoline is a biological alkylating agent by virtue of its ability to undergo addition reactions across the reactive Δ^3-ethylenic bond; *N*-acetyl-*S*-(3-carboxy-1-methylpiperid-4-yl)-L-cysteine has been detected in the urine of rats which received the drug by intraperitoneal injection or in the drinking water (Boyland & Nery, 1969). The present work demonstrates that arecoline, like many other drugs having a *tert.*-amino structure (e.g. nicotine, morphine, schradan and tremorine; see the review by Bickel, 1969), is metabolized by 1-oxide formation. 1-Oxidation of tertiary amines occurs in the microsomal system of liver requiring NADPH and oxygen, although this may not be the microsomal system involving cytochrome *P*-450 and NADPH–cytochrome *c* reductase, which oxidizes drugs and other lipophilic substances (Ziegler, Pettit & Orme-Johnson, 1965). The effect of the *N*-oxidation of *tert.*-amine drugs is often to increase the biological action of the drug, e.g. the carcinogenic action of 4-aminoquinoline (Arai & Nakayama, 1952) and 4-nitroquinoline (Kawazoe, Tachibana, Aoki & Nakahara, 1967). Although only 3–4% of the administered arecoline appeared as 1-oxides in the urine (see Table 1), the true extent of 1-oxide formation in the body was probably much higher because of the reduction *in vivo* of arecoline 1-oxide to arecoline. Many other *tert.*-amine 1-oxides, including pyridine 1-oxide (May, 1957) and purine *N*-oxides (Dunn, Maguire & Brown, 1959), are similarly reduced.

In the rat, arecoline is metabolized by three routes: (*a*) ester hydrolysis (reaction I→II, Scheme 1), (*b*) 1-oxidation together with (I→IV) or without (I→III) ester hydrolysis and (*c*) addition across the Δ^3-ethylenic bond together with ester hydrolysis (I→V).

The chemical oxidation of arecoline by peroxyacetic acid proceeded in two stages: 1-oxidation preceded epoxidation of the Δ^3-ethylenic bond. In

ethereal solution, the 1-oxide separated out as it was formed and epoxidation did not occur; in acetone or aqueous solution, in which the primary product of oxidation, i.e. the 1-oxide, was soluble, epoxidation also occurred. Arecaidine, probably because of the electrophilic nitrogen atom of the zwitterionic structure of the compound in aqueous solution between pH 5.4 and pH 6.8 (Boyland & Nery, 1969), was oxidized less readily than arecoline by aqueous hydrogen peroxide. The structure of arecoline 1-oxide was established by (i) elemental analysis, (ii) deoxygenation with triethyl phosphite to regenerate arecoline (reaction III→I, Scheme 1), (iii) acid hydrolysis to yield arecaidine 1-oxide (III→IV) and thermal decomposition to give 1,2-dihydro-1-methylnicotinic acid methyl ester (III→VI), probably via rearrangement to the 6-hydroxy derivative followed by loss of the elements of water. Compound VI condensed with 4-dimethylamino-cinnamaldehyde to give the highly conjugated dye VIII, the colour of which resulted from resonance structures of the types shown.

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