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Chemical and Pharmacological Evaluation of *Banistereopsis argentea* Spring ex Juss

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Abstract □ Seven simple indole bases—viz., *N,N*-dimethyltryptamine (I), its *N*-oxide, harmine (II), harmaline (III), (+)-*N*-methyltetrahydroharman (IV), (+)-tetrahydroharman (V), and (+)-5-methoxytetrahydroharman (VI)—were isolated from the leaves and stems of *Banistereopsis argentea* Spring ex Juss (Malpighiaceae) and their identity established by physical and chemical methods. In addition to these alkaloids, two partially characterized minor indole-3-alkylamines, together with choline and betaine, were obtained from the same parts of the plant. This was the first demonstration of the occurrence of *N*-methyltetrahydroharman in the family Malpighiaceae and of a 5-methoxytetrahydro- β -carboline alkaloid in nature. The total alkaloids from the leaves have shown three main pharmacological actions: nonspecific spasmolytic action against acetylcholine-, histamine-, and serotonin-induced spasm; histamine release-mediated depressor response; and pronounced behavioral effects.

Keyphrases □ *Banistereopsis argentea* Spring ex Juss—isolation, identification, pharmacological evaluation of seven indole alkaloids □ *N*-Methyltetrahydroharman—isolation, identification from *B. argentea* □ 5-Methoxytetrahydro- β -carboline alkaloid—occurrence in nature, *B. argentea* □ CNS activity—indole alkaloids in *B. argentea* □ TLC—separation, isolation, *B. argentea* alkaloids □ Mass spectrometry—identification, structure

The use of members of the genus *Banistereopsis* (family Malpighiaceae) in the preparation of narcotic and hallucinogenic decoctions by certain Colombian and Peruvian Indian tribes is well known (1). Earlier chemical investigations with a number of *Banistereopsis* species showed (2-5) the presence of the following four indole bases in their various parts: *N,N*-dimethyltryptamine (I), harmine (II), harmaline (III), and (+)-tetrahydroharman (V). Since then, there has been much

speculation (6, 7) as to the contribution of the individual alkaloidal entities to the hallucinogenic activity of the crude preparations. Although the hallucinogenic activity of harmine was demonstrated earlier, the crude preparations of plants containing harmine have, however, appeared to produce greater psychotropic action than pure harmine (6). Moreover, the well-documented hallucinogenic activity of *N,N*-dimethyltryptamine (7) cannot alone account for the strong behavioral changes caused by the plant extracts, because the indole base is only present as a minor constituent in the genus *Banistereopsis*. It seems likely that the strong hallucinogenic activity of the crude plant preparations is due to some unidentified, more potent, extraneous materials introduced or generated chemically during preparation, to other natural products, or to a synergistic action of the mixture.

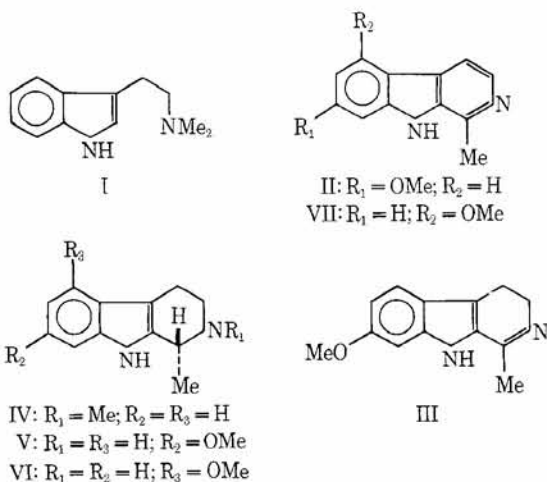
To extend the knowledge of the content and distribution of the aforementioned and related alkaloids in the genus *Banistereopsis*, the phytochemical and pharmacological evaluation of a rare species, *B. argentea* Spring ex Juss¹, was undertaken. Some recent investigations were directed toward the study of Indian medicinal plants used as psychotherapeutic agents in the Ayurvedic system of medicine. The results, with a number of species belonging to the families Leguminosae and Gramineae, showed the presence of about two dozen indole-3-alkylamines and β -carbolines, several of which were either hallucinogens or MAO inhibitors (8-14). The

¹ The plant material was obtained through Mr. R. Biswas, Indian Botanic Gardens, Howrah, India.

present paper deals with isolation, identification, and pharmacological studies of alkaloids from the stems and leaves of *B. argentea*.

CHEMISTRY

From the leaves of the plant, seven indole-3-alkylamine and β -carboline bases have been isolated to date. These are identified as *N,N*-dimethyltryptamine (I) and its *N*-oxide, harmine (II), harmaline (III), (+)-*N*-methyltetrahydroharman (IV), (+)-tetrahydroharman (V), and a new tetrahydro- β -carboline alkaloid, (+)-5-methoxytetrahydroharman (VI). The structure of VI was established from its color reactions with alkaloidal reagents; determinations of optical rotation, UV, and mass spectra; and dehydrogenation with palladium-on-charcoal to 5-methoxyharman (VII) which had been confirmed by comparison with a synthetic sample.



This was the first demonstration of the occurrence of a 5-methoxy-tetrahydro- β -carboline (VI) in nature and of (+)-*N*-methyl-tetrahydroharman in the family Malpighiaceae. The new naturally occurring base, which was obtained in the (+)-form, was tentatively assigned the R-configuration by analogy with the established steric configuration of (+)-tetrahydroharman (V) (15) and (+)-tetrahydroharman (IV) (16).

In addition to the aforementioned alkaloids, two partially characterized quaternary indole-3-alkylamines and a liberal amount of choline and betaine were isolated from the leaves of this plant. The stems contain the same alkaloids but in poorer yields.

The presence of both 5- and 7-methoxytetrahydro- β -carbolines in a single plant species such as this is of considerable biogenetic and chemotaxonomic significance. In view of the currently accepted hypothesis regarding the genesis of hydroxy- (or methoxy)-indole-3-alkylamines, involving *N,N*-dimethyltryptamine (17, 18), it would seem likely that the corresponding hydroxylation in β -carbolines, derived from tryptophan (or tryptamine) (19), would take place after the piperidine-pyridine ring (C-ring) formation.

PHARMACOLOGY

Detailed pharmacological study was made only with the total alkaloids (consisting of Fractions A, B, and D). The crude plant extracts elicited only feeble activity, while the individual bases were not available in quantities sufficient for complete screening. The behavioral effects were, however, studied with both total alkaloids and with the tetrahydro- β -carbolines (obtained from Fraction B).

Effect on Smooth Muscles—Guinea Pig Ileum—The total alkaloids (10–20 mcg./ml.) produced an initial spasm, which was followed by tachyphylaxis after a second or third dose of the drug. In the same concentrations, the drug showed spasmolytic activity against equispasmodic doses of acetylcholine (0.1 mcg./ml.), histamine (0.1 mcg./ml.), and serotonin (0.5 mcg./ml.). The antagonism followed the order: acetylcholine < histamine < 5-hydroxytryptamine. The initial inhibition of the spasmogens was subse-

quently followed by marked potentiation. In case of the tetrahydro- β -carbolines, more powerful antagonism to serotonin and subsequent potentiation were observed.

Rat Uterus—The total alkaloids (20–50 mcg./ml.) elicited spasm in isolated estrus uterus of the rat. The spasm was antagonized by pretreatment of the isolated tissue with lysergic acid diethylamide (1 mcg./ml.). In smaller concentrations (0.2–0.5 mcg./ml.), the drug caused facilitation of the serotonin response.

Effect on Skeletal Muscle—Frog Rectus Abdominis Muscle—The total alkaloids and those from Fraction D blocked specifically acetylcholine-induced (1 mcg./ml.) spasm in isolated frog rectus abdominis muscle. The drug did not alter the KCl-induced (3 mg./ml.) spasm. The antagonism to acetylcholine was reversible; the spasmolytic ED_{50} 's were 35 (for total alkaloids) and 8.5 (for alkaloids from Fraction D), bufotenidine control (11) being used.

Effect on Heart—Frog Heart—No significant action was observed with the total alkaloids (up to a dose of 200 mcg./ml.) on perfused frog heart preparation.

Effect on Blood Pressure, Respiration, and Intestine In Situ—The total alkaloids (0.5–1.0 mg./kg. i.v.) produced an initial transient rise and then a sharp fall in blood pressure of the pentobarbital sodium- (35 mg./kg. i.p.) anesthetized dog. The recovery was slow and gradual. Marked bronchoconstriction was observed, which was preceded by a transient respiratory stimulation. In dogs pretreated with polysorbate 80 (0.01 ml./kg.), the depressor response and bronchoconstriction were very much less marked. These observations indicated that the depressor effect was histamine-release mediated (11, 13). When the respiration was artificially maintained, the total alkaloids (1–2 mg./kg.) produced a pressor response which was followed by tachyphylaxis on repeated drug administrations. The pressor response was antagonized by tolazoline² (10 mg./kg.) and was absent in reserpinized dogs. The pressor response was, therefore, catecholamine-release mediated.

The intestinal tone was increased during the depressor response; in artificially respired dogs, intestinal relaxation was followed by tachyphylaxis on repeated drug administrations.

Effect on Frog Melanophores—Histamine-releasing activity of the total alkaloids was studied according to the method of Bhidé and Gupta (20). *In vivo* administration of the drug (0.5–1.0 mg./kg.) produced, within 15–20 min., yellow coloration of the dorsal skin, intense red coloration of the ventral skin, and punctation of melanophore granules in the web melanophores. The effect was over within 60–90 min. In the case of the water-soluble alkaloids (Fraction D), the histamine-releasing activity was more pronounced and the changes were caused by much lower concentrations (0.2–0.4 mg./kg.).

Hallucinogenic Activity—The initial testing for the psychotropic activity of the total alkaloids and of the tetrahydro- β -carbolines (from Fraction B) was done on estrus rat uterus according to the method of Costa (21)³. The test compounds, in low concentrations (0.1–0.2 mcg./ml.), caused, in varying degrees, direct potentiation of serotonin-induced spasm. In higher concentrations, only the total alkaloids caused an initial antagonism to serotonin, while the tetrahydro- β -carbolines had no significant effect on serotonin response.

The total alkaloids, in low concentrations (1–3 mg./kg. i.p.), produced excitation, fine tremors, salivation, and hypermotility in mice and rats. In higher concentrations (5–10 mg./kg.), the drug caused jumping movements, rapid tapping of the forelimbs, paralysis of the hindlimbs, convulsions, and respiratory arrest. In the case of 5-methoxy-*N,N*-dimethyltryptamine, these effects were observed in similar concentrations. A laparotomy, after the respiratory arrest, showed that the heart was still beating although the lungs had collapsed.

These effects, with the exception of the hindlimb paralysis in rats (which might be partly associated with the neuromuscular blocking activity of the alkaloids), were almost completely antagonized by pretreatment with chlorpromazine (5 mg./kg. s.c.) but not by atropine (100 mg./kg.). The effects, in the case of the total alkaloids, were potentiated by nialamide (10 mg./kg.), a known MAO inhibitor. Further, the total alkaloids also antagonized chlorpromazine- and reserpine-induced (5 mg./kg.) sedation in mice.

² Prisol.

³ Costa (21) reported that the facilitation of the spasmogenic activity of serotonin on rat uterus by a drug, in low concentration, was a typical effect of compounds that cause hallucination, e.g., mescaline and lysergic acid diethylamide.

EXPERIMENTAL⁴

The general procedure for the isolation of the alkaloids involved gradient-pH extraction of the CHCl_3 -soluble bases; gradient-pH precipitation of the reineckates of water-soluble bases (22); regeneration of water-soluble bases by passing the reineckate salts in acetone or alcohol over De-Acidite FF resin column (pH 8); and preparative chromatography over neutral alumina (S. Merck, according to Brockmann specification) and magnesia-silica gel⁵ columns and over silica gel G chromatoplates. Identification of the individual entities involved determination of melting point; mixed melting point; co-TLC in the presence of marker compounds; UV, IR, and mass spectra; and preparation of picrate, hydrochloride, and methiodide where possible. In the TLC, methyl alcohol- CHCl_3 -acetic acid (75:25:15) was used as the developer; Dragendorff (D), Ehrlich (E), and α -nitroso- β -naphtholnitrous acid (N) reagents were used for the staining purposes. Indole-3-alkylamines and tetrahydro- β -carbolines showed light-blue fluorescence under UV light on papers, while aromatic β -carbolines showed violet and 3,4-dihydro- β -carbolines showed sea-green fluorescence. None of the alkaloids from this investigation responded to the Jepson and Stevens test (23) for N_5 -unsubstituted tryptamines.

Isolation of Alkaloids—Dried and milled leaves (1.8 kg.) were defatted with petroleum ether (60–80°) and then extracted with ethyl alcohol in a Soxhlet for 16 hr. The ethyl alcohol extract was concentrated under reduced pressure to give a viscous brown consistency (78 g.), which was poured into 2% acetic acid (200 ml.) with stirring. The mixture was kept at ordinary temperature overnight. Suspended impurities were filtered off; the clarified solution was shaken with CHCl_3 (three 50-ml. portions), which removed the weakly basic CHCl_3 -soluble acetates (11 g.) (Fraction A). The aqueous acidic solution was basified with NH_4OH , and the liberated bases were extracted with CHCl_3 (150 ml.). The CHCl_3 extract was washed (H_2O) and dried (CaCl_2), and the solvent was removed under reduced pressure. The residue (4.2 g.), a brown basic gum, was marked Fraction B. The aqueous alkaline mother liquor was treated with a saturated aqueous solution of ammonium reineckate, and the light-pink reineckate salt (2.7 g.) was marked Fraction C. The filtrate was acidified (6 N H_2SO_4), and more ammonium reineckate solution was added to ensure complete precipitation of the complex salt. The reineckate salt (1.8 g.), obtained under acidic conditions, was marked Fraction D.

Fraction A—The residue from this fraction, containing a mixture of alkaloids, was dissolved in benzene and chromatographed over alumina (35 $\times$ 2.5 cm.). Elution was carried out with petroleum ether, benzene, CHCl_3 , methyl alcohol, and different proportions of mixtures thereof. Forty-milliliter fractions were collected.

N_5 -Methyltetrahydroharman (IV)—The first few fractions of the CHCl_3 eluates gave a pale-yellow oil (37 mg.); R_f 0.51; D, orange, E and N, negative; $[\alpha]_D^{25} + 26^\circ$ (c, 0.72; CHCl_3); λ_{max} , 225–228, 278, and 293 nm. (log ϵ , 4.50, 3.83, and 3.81); m/e 200 (M^+ , 58%), significant peaks at m/e 199 (100) and 197 (72); yellow picrate from ethyl alcohol- H_2O as needles, m.p. 181–183°; the identity was established by superimposing IR spectra of the base with an authentic specimen and also by determining the mixed melting point of the picrate, which was not depressed.

N,N -Dimethyltryptamine (I)—The CHCl_3 -methyl alcohol (99:1) eluates gave N,N -dimethyltryptamine as a brown oil (48 mg.); R_f 0.47; D, orange; E, blue; N, negative; λ_{max} , 224, 274, and 294 nm. (log ϵ , 4.48, 3.77, and 3.75); as yellowish-orange picrate needles from methyl alcohol- H_2O , m.p. 166–167°; as colorless methiodide needles from acetone-methyl alcohol, m.p. 216°; mixed melting point of the derivatives with authentic specimens remained undepressed.

N,N -Dimethyltryptamine- N_5 -oxide—The *tert*-amine oxide migrated from the column as a hygroscopic solid (26 mg.) with methyl alcohol eluates; R_f 0.88; D, dirty orange; E, cherry red; N, negative; reduction with Zn dust and acetic acid gave N,N -dimethyltryptamine (co-TLC, mixed melting point of the picrate).

Fraction B—The basic gum showed six Dragendorff-positive spots, R_f 0.32–0.95. The gum was dissolved in 1 N HCl (60 ml.), and the acidic solution was extracted with ethyl acetate (three 50-ml. portions). The organic layer was washed and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue (1.3 g.) was dissolved in benzene and chromatographed over Florisil (50 g.) using CHCl_3 , CHCl_3 -methyl alcohol, and methyl alcohol as eluents.

Harmine (II)—The middle CHCl_3 eluates afforded a brown amorphous solid (0.8 g.); R_f 0.73 and 0.86. The major base crystallized from methyl alcohol- H_2O as colorless needles, m.p. 258–260°, mixed melting point with an authentic synthetic sample of harmine (m.p. 260–261°) remained undepressed; R_f 0.73; D, orange; E, negative; N, faint violet; λ_{max} , 240, 303, 323, and 338 nm. (log ϵ , 4.61, 4.20, 3.62, and 3.68). The base hydrochloride crystallized from ethyl alcohol as colorless needles, m.p. and mixed m.p. 268–270°.

The mother liquor, after separation of harmine hydrochloride (ethyl acetate), was evaporated to dryness. The residue was taken in H_2O (10 ml.) and basified (NH_4OH). The liberated bases were extracted with CHCl_3 ; the CHCl_3 extract showed four Dragendorff-positive spots on TLC. The extract was evaporated to dryness, and the residue was dissolved in benzene (12 ml.) and chromatographed over Florisil (38 g.). CHCl_3 and CHCl_3 with increasing proportions of methyl alcohol were used as eluents. The first three fractions of the CHCl_3 eluates afforded a further crop of harmine (72 mg.). The CHCl_3 -methyl alcohol (1:1) eluates gave a brown gum (97 mg.) consisting of two bases, R_f 0.62 and 0.66.

(+)-Tetrahydroharman (V)—The above-mentioned basic gum was dissolved in xylene containing traces of ether; the solution, on keeping at ordinary temperature, gave (+)-tetrahydroharman as straw-colored microneedles, m.p. 195–198° (16 mg.) [lit. (24) m.p. 199°]; R_f 0.66; D, orange; E, negative; N, dull violet; Fröhde, navy blue; Hopkin-Cole's glyoxalic reagent, purple; $[\alpha]_D^{25} + 28^\circ$ (c, 0.516; CHCl_3); λ_{max} , 217 and 268–272 nm. (log ϵ , 4.53 and 3.72); m/e 216 (M^+ , 42%), significant peaks at m/e 215 (100), 213 (47), 200 (52), 197 (18), 170 (14), and 169 (52).

(+)-5-Methoxytetrahydroharman (VD)—The xylene-ether mother liquor showed two spots, R_f 0.62 (major component) and 0.66 (traces). The two components were separated by preparative TLC on H_2SO_4 (5%) treated silica gel G chromatoplates. The major component was obtained as a pale-brown gum (66 mg.), and the corresponding base was obtained in the usual way. It could not be induced to crystallize. The base showed alkaloidal color reactions typical of tetrahydro- β -carbolines: Fröhde, navy blue; Hopkin-Cole, purple; $[\alpha]_D^{25} + 34^\circ$ (c, 0.83; CHCl_3); λ_{max} , 215–217 and 290 (sh) nm. (log ϵ , 4.55 and 3.66); m/e 216 (M^+ , 72%), significant peaks at m/e 215 (100), 213 (16), 201 (35), and 169 (64).

Dehydrogenation of (+)-5-Methoxytetrahydroharman—A suspension of 10% palladium-on-charcoal catalyst (0.1 g.) and the alkaloid (24 mg.) in xylene (40 ml.) was refluxed for 8 hr., and the mixture was filtered hot. The solvent was removed from the filtrate under reduced pressure. The amorphous brown residue (19 mg.) showed λ_{max} , 242–244, 286, 332, and 344 nm. (log ϵ , 4.62, 4.03, 3.71, and 3.73); m/e 212 (M^+ , 100%), significant peaks at m/e 197 (16) and 169 (84), metastable peak observed at m/e 145 (197 $\rightarrow$ 169 transition requires m^* at m/e 145). The methosulfate crystallized from acetone-methyl alcohol as needles, m.p. 285° [lit. (24) m.p. 285°]; λ_{max} , 248–252, 288, 338, 342, and 358 nm. (log ϵ , 4.59, 4.11, 3.68, 3.71, and 3.71); λ_{max} , (ethyl alcohol-NaOH) 255, 305, and 340 nm. (ϵ not accurate). The UV spectral data are in excellent agreement with those reported in the literature (24).

Harmaline (III)—The methyl alcohol eluates (from Fraction B) on evaporation gave a brown solid (21 mg.), which was further purified by sublimation *in vacuo*. The pale-yellow prisms sublimed at 110–120°/0.01 mm. and had a melting point of 236–238°. Mixed melting point with an authentic sample of harmaline⁶ (m.p. 236–238°) remained undepressed. The identity of the alkaloid as harmaline was further established from co-TLC, R_f 0.38; m/e 214 (M^+), and by superimposing the IR spectra on the spectrum of an authentic specimen.

Fraction C—The reineckate complex was dissolved in ethyl alcohol (25 ml.) and was passed through a column of De-Acidite FF (pH 8). Elution was done with the same solvent. The total eluates (120 ml.), on evaporation under reduced pressure, gave a hygroscopic

⁴ Melting points were determined on a Toshniwal melting-point apparatus, in open capillary, and are uncorrected. UV spectra were recorded in a Carl Zeiss PMQ II spectrophotometer in spectral EtOH. IR spectra were recorded with a Perkin-Elmer 137 instrument in KBr and mineral oil. Mass spectra were determined with an AEI MS 9 instrument operated at 70 eV. Microanalyses were performed by the Analytical Section, Central Drug Research Institute, Lucknow.

⁵ Florisil.

⁶ The authors are grateful to Prof. (Mrs.) A. Chatterjee, Calcutta University, for an authentic specimen of harmaline.

solid (0.17 g.); R_f 0.12; D, pink; E and N, negative. The base picrate crystallized from ethyl alcohol as orange-yellow needles, m.p. 242°, mixed melting point with authentic choline picrate (m.p. 242°) remained undepressed. Usual pharmacological testing (25) with a portion of the base also showed its identity with choline.

Fraction D—The reineckate salt, precipitated under acidic conditions, was treated in the same manner; a brown basic gum (82 mg.) was obtained. The gum showed three Dragendorff-positive spots on TLC, R_f 0.09, 0.12, and 0.28.

Betaine—An ethyl alcohol solution of the above base was treated with a solution of picric acid in the same solvent, and the mixture was concentrated. Betaine picrate separated as yellow needles, m.p. and mixed m.p. 188–190°.

The ethyl alcohol mother liquor, after separation of betaine picrate, was passed through a column of De-Acidite FF. The regenerated mixture of bases was separated by preparative TLC.

Quaternary Indole-3-alkylamines—The major component (R_f 0.09; D, orange, E, blue; N, violet) showed UV λ_{max} 218, 272 (sh), and 285 nm., characteristic of indole-3-alkylamines. The minor component, (R_f 0.12; D, orange; E, blue; N, dull violet) showed λ_{max} 224–226, 272, 294, and 305–310 (sh) nm., characteristic of 5-oxy-indole-3-alkylamines.

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Degradation of Urea in Concentrated Aqueous Solution

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Abstract □ The degradation of urea in 2.00, 4.00, 6.00, and 8.00 *M* aqueous solutions was studied at 25.0, 35.0, and 45.0°. Data were obtained by measuring the specific conductivity of the solutions at 6-hr. intervals over 3.5 days. The results show that the degree of degradation is extremely small and that the overall process conforms to a first- and second-order reversible reaction. Rate constants were determined for the forward and reverse reactions and compare favorably to values reported by other workers for the separate reactions.

Keyphrases □ Urea—degradation in concentrated aqueous solution, temperature control □ Conductivity—urea degradation monitoring □ Rate constants—aqueous urea degradation □ Specific conductivity—urea degradation monitoring

The hydrolysis of urea in various aqueous media has been studied extensively. An excellent review and bibliography on the subject are given by Frost and

Pearson (1). However, these earlier investigations, including a more recent study by Lynn (2), were concerned with the nature of the hydrolysis reaction as it occurs in relatively dilute solutions of urea and, for the most part, at temperatures between 60 and 100°. As a consequence, little information exists relative to the degradation process under conditions of current interest. Thus, workers who are studying solubility (3–5) and denaturation (6, 7) phenomena in solutions ranging up to 9 *M* in urea, and at temperatures between 25 and 50°, are unable to appraise the untoward effects produced by this reaction. Instead, and because degradation is known to occur, investigators are compelled to initiate their studies with freshly prepared urea solutions in the hope that these effects will be minimized. Since solubility studies, for example, characteristically require days for equilibration, even this technique offers little in the way of reduced uncertainty.