employed is one where death is delayed for more than 1 week and is ascribable to hematopoietic depression rather than to CNS effects. Mortality in the experiments of Laird and Fonner occurred following exposures in the 10,000-r range 2–3 days postirradiation and was associated with convulsions, which was not the case in this experiment. Furthermore, DPH was given in this experiment in a single injection at a dose level one-fourth the anticonvulsant dose in mice (9) and would certainly not be expected to exert any anticonvulsant activity 10 days following drug administration.

Potentially important nonanticonvulsant actions of the DPH drug group have been identified by Gordon *et al.* and include antileukemic activity (5) and enhancement of the deteriorated learning and memory characteristic of very old animals (10, 11). Furthermore, both DPH and pemoline have been identified by this group as markedly potentiating the activity of the enzyme DNase 1, while DPH has been found by Shafer (12) to increase markedly the turnover of DNA in normal liver. Thus, DPH may exert the radioprotective action reported here by effects on nucleic acid metabolism, which are in no simple way related to its anticonvulsant action on the brain.

Continuing investigations will include exploring the capacity of DPH to exert radioprotective effects when given after radiation because such an effect for DPH has been observed in these laboratories when it is given within the 1st hour. Further, in a series of preliminary experiments, DPH exerted an effect similar to that of pemoline (5-phenylpseudohydantoin) in altering the growth pattern of Ehrlich carcinoma while prolonging the lifespan of tumor-bearing animals (13–15). The fact that DPH and pemoline have a similar chemical structure and exert similar effects on a nucleic acid-metabolizing enzyme suggests the link between biological effects shared by these two compounds.

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Erythrina sp. III: Chemical Constituents of *Erythrina suberosa* Roxb. Seeds

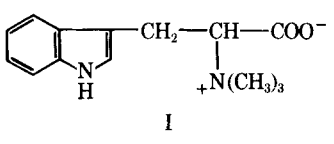
HARKISHAN SINGH and AMRIK SINGH CHAWLA

Abstract □ A phytochemical investigation of *Erythrina suberosa* seeds has resulted in the isolation of erythraline, erysodine, erysotrine, and hypaphorine. This is the first time that erysotrine has been found to occur naturally, although it is well known as a conversion product of other eryso-alkaloids. The alkaloidal constituents were found to vary in different seed collections. The fatty acid composition of the seed oil was examined, and the sterol part from the unsaponifiable matter was found to be composed of sitosterol, stigmasterol, campesterol, and cholesterol.

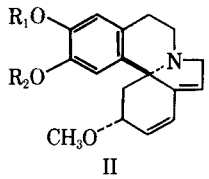
Keyphrases □ *Erythrina suberosa* seeds—phytochemistry, chemical constituents □ Mass spectroscopy—identification □ IR spectrophotometry—identification □ TLC—identification

In India, *Erythrina stricta* Roxb., *E. suberosa* Roxb., and *E. variegata* Linn. var. *orientalis* (Linn.) Merrill (syn. *E. indica* Lam.) have been used in the indigenous system of medicine for various ailments (1).

The authors have started a systematic study of *Erythrina* species growing in India. There have been no earlier reports of chemical investigations of *E. suberosa* and *E. stricta*. Hypaphorine (I) has been isolated from the seeds of *E. variegata* var. *orientalis* (2–6), and Folkers and Koniuszy (6) obtained erythraline



I



II

(II; $R_1,R_2 = -CH_2-$) from the seeds. Subbaratnam (5) isolated a neutral entity, $C_{24}H_{50}O_2$, m.p. 82–84°. The fatty acids present in the seeds have been studied (3, 7). The bark of *E. variegata* var. *orientalis* has been investigated by various workers (3, 8, 9), and preliminary studies on the leaves (3, 10) have been made.

Recently, the present authors fractionated the petroleum ether extract of *E. suberosa* bark into wax esters, alcohols, and acids; alkyl ferulates; and stigmasterol, sitosterol, campesterol, and cholesterol (11). In this paper, results from an investigation on the chem-

Table I—Contents of Fatty Acids Present in *E. suberosa* Seed Oil

Fatty Acid	Percent Weight	Percent Mole
Myristic acid	Traces	
Palmitic acid	12.2	13.4
Stearic acid	2.4	2.4
Oleic acid	38.3	39.2
Linoleic acid	24.7	25.4
Arachidic acid	3.5	3.2
Eicosenoic acid	4.5	4.4
Behenic acid	11.3	9.6
Docosenoic acid	Traces	
Lignoceric acid	3.1	2.4

ical constituents of *E. suberosa* seeds are reported. Three collections, made from Dehradun, Mandi, and Joginder Nagar in India, have been examined; a preliminary communication about the alkaloidal constituents of the seeds has been published (12).

From the powdered seeds, the oil was separated through extraction with petroleum ether. The physical and chemical characteristics of the oil and the chemical characteristics of the separated fatty acids were determined. On spectrophotometric estimation, the oil revealed the presence of 22.9% of diene acids calculated as linoleic acid, 43.7% of mono-ene acids calculated as oleic acid, and 33.4% of saturated fatty acids (found by difference). The fatty acid content in terms of percent weight and percent mole as estimated by GLC of the methyl esters is listed in Table I.

The unsaponifiable matter of the seed oil yielded a sterol material, which the GLC of the trimethylsilyl (TMS) ethers showed to be a mixture of sitosterol (67%), stigmasterol (20%), campesterol (12%), and cholesterol (1%).

The Dehradun seed powder was defatted with petroleum ether and the marc extracted with ethanol. The petroleum ether extract yielded an alkaloidal part (Fraction A). The ethanol extract gave a chloroform-soluble part (Fraction B), which on chromatographic resolution gave a crystalline base identified as erythraline (II; R_1,R_2=—CH_2—). Fraction A, which was resinous, gave erythraline hydriodide. The aqueous alkaline layer remaining after removal of Fraction B from the ethanol extract was acidified, refluxed, and extracted with chloroform to give Fraction C, which did not yield crystalline material. The aqueous layer yielded a water-soluble base identified as hypaphorine (I).

The Mandi seed collection gave Fraction D from the petroleum ether extract, and Fractions E and F from the ethanol extract, representing "free" and "liberated" alkaloidal parts. Fraction D yielded a crystalline hydrochloride which appeared to be erysotrine (II; $R_1 = R_2 = CH_3$) hydrochloride according to elemental and UV data. Fraction F gave crystalline erysodine (II; $R_1 = H$, $R_2 = CH_3$). Contrary to earlier observations (13), it was possible to prepare the hydrochloride of the isolated erysodine. Chromatogaphy of Fraction E led ultimately to crystalline erysotrine hydrochloride and erysodine. A confirmation of the identity of isolated erysotrine hydrochloride came from a mass spectrum of the liberated base from the salt. The base indicated significant peaks at *m/e* 313 (molecular ion M^+; 33% intensity of base peak), 298 (M^+ —CH_3; 35%), and 282 (M^+ —CH_3O; base peak), which compared with the authentic specimen. Hypaphorine was also isolated from the Mandi seeds.

Because of the alkaloidal variations found in the Dehradun and Mandi collections of *E. suberosa* seeds, a third collection, from Joginder Nagar, was investigated. These seeds also yielded the "free" bases erysotrine and erysodine but no erythraline.

The difference in the alkaloidal variations mentioned may be attributed to differences in climatic or soil conditions, and the two plants possibly may belong to different strains. The other aspect of interest is the isolation of erysotrine because never before has erysotrine been found to occur naturally, although it is well known as a conversion product of other erysoalkaloids.

EXPERIMENTAL

Plant Material[1]—The seeds were procured[2] from Dehradun (Uttar Pradesh, India), and also collected from Mandi and Joginder Nagar (Himachal Pradesh, India). The seeds were reduced to moderately coarse powder.

Extractives—The seed powder (Dehradun) (25 g.) was extracted successively in a continuous extraction apparatus, and the percent extractives was determined: petroleum ether (60–80°), 8.10%; benzene, 0.51%; ether, 0.07%; chloroform, 0.64%; acetone, 0.84%; ethanol, 18.20%; and water, 5.30%.

Extraction and Study of Oil—The seed powder (Dehradun) (500 g.) was extracted with petroleum ether (60–80°) for 15 hr. to yield 40 g. of the oil, which had the following characteristics: sp. gr. 25°/25°, 0.9483; n_D^{25} 1.4690; iodine value, 74.6; acid value, 8.6; saponification value, 173; and unsaponifiable matter, 1.6%.

The mixed fatty acid fraction (iodine value, 80.9; saponification equivalent, 281) obtained from the oil was found to have a total diene composition and mono-ene composition of 22.9 and 43.7%, respectively, as determined by the alkali isomerization method of Hilditch *et al.* (14) and using reference values of Hilditch *et al.* (15). By difference the saturated fatty acids come to 33.4%.

The mixed fatty acids were converted to their methyl esters (16). The IR spectrum of the methyl esters showed bands at 2997 cm.$^{-1}$ (olefinic C—H stretching); 2918 and 2846 cm.$^{-1}$ (for CH_2 and CH_3); 1740 cm.$^{-1}$ (C=O stretching); and 1230, 1185, and 1160 cm.$^{-1}$ (C—O stretching for esters).

The methyl esters were analyzed by GLC on a polyester column [20% diethylene glycol succinate on diatomite[3] 2.44 m. (8 ft.) × 0.49 cm. (0.19 in.), 215°] with a thermal conductivity detector. The peak areas were measured by triangulation and the results are given in Table I.

The unsaponifiable part of the seed oil was separated in the usual way. A chromatographic resolution on an alumina column gave a sterol fraction, m.p. 139°. The IR spectrum showed a band at 3440 cm.$^{-1}$ (broad, associated O—H stretching) and a weak but very diagnostic peak at 972 cm.$^{-1}$ for the *trans*-disubstituted double bond of stigmasterol.

The sterol fraction, when resolved as its TMS ether on an SE-30 column [1% on Anakrom SD, 3.04 m. (10 ft.) × 0.32 cm. (0.125 in.), 260°], proved to be a mixture of sitosterol (67%), stigmasterol (20%), campesterol (12%), and cholesterol (1%). A standard mixture of TMS ethers of the corresponding sterols was used for identification of the peaks.[4]

Isolation and Detection of Alkaloids—Dehradun, Mandi, and Joginder Nagar seeds were investigated separately.

[1] The authenticity of the seeds was certified by Dr. T. S. Sareen, Curator, Department of Botany, Panjab University.
[2] Through M/s Pratap Nursery and Seed Stores, Dehradum.
[3] Chromosorb W, Johns-Manville.
[4] These data were obtained through Dr. J. W. Rowe, USDA Forest Products Laboratory, Madison, Wis.

Dehradun Seeds—The seed powder (2.0 kg.) was extracted with petroleum ether (60–80°) in a continuous extraction apparatus. The basic components were removed from the petroleum ether extract by shaking with 2% w/v sulfuric acid. The acid layer (1 l.) was made alkaline with a dilute ammonia solution (150 ml.) and extracted with chloroform. The chloroform extract was worked up to give a residue (0.93 g., Fraction A).

The marc was extracted with ethanol in a continuous extraction apparatus. The residue (300 g.) remaining after evaporation of the solvent was repeatedly treated with 2% w/v sulfuric acid. The acid layer (2 l.) was washed with chloroform (3 × 300 ml.) to remove the nonalkaloidal matter. The acid extract was made alkaline with a dilute ammonia solution (350 ml.) and extracted with chloroform (10 × 300 ml.). The chloroform extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to yield a residue (5.7 g., Fraction B).

Fraction B was chromatographed over alumina (Merck, 170 g.). Elution with benzene gave a sticky solid mass (3.0 g.), which was repeatedly crystallized from absolute ethanol to give a base, m.p. 111–113° [lit. (6) erythraline, m.p. 106–107°].

Anal.—Calcd. for $C_{18}H_{19}NO_3$: N, 4.71. Found: N, 5.11. $[\alpha]_D^{20}$ + 192.5° (c, 0.78, $CHCl_3$) [lit. (6) $[\alpha]_D^{27}$ + 211.8° (c, 0.944, EtOH)]; UV $\lambda_{max.}^{MeOH}$ 292 mμ (log ε 3.6) [lit. (17) 292 mμ (log ε 3.6)].

The base showed no depression on determining mixed melting point with authentic erythraline. The base formed a hydrobromide, m.p. 250–251° dec. [lit. (17) erythraline hydrobromide, m.p. 246°].

Anal.—Calcd. for $C_{18}H_{19}NO_3 \cdot HBr$: C, 57.16; H, 5.33; Br, 21.13; N, 3.70. Found: C, 57.47; H, 5.30; Br, 21.25; N, 3.56. UV $\lambda_{max.}^{MeOH}$ 290 mμ (log ε 3.6). The IR spectra of the base hydrobromide and authentic erythraline hydrobromide were identical.

The hydrochloride of the base was prepared in the usual way and crystallized from dry ether–absolute ethanol to give an entity, m.p. 251–252° dec.

Anal.—Calcd. for $C_{18}H_{19}NO_3 \cdot HCl$: C, 64.79; H, 6.04; Cl, 10.63; N, 4.20. Found: C, 64.66; H, 6.64; Cl, 10.80; N, 4.19.

Fraction A was not resolvable on an alumina column. Its hydriodide, m.p. 247–248° dec., was prepared and showed no depression in melting point on admixture with the hydriodide of erythraline isolated from Fraction B [lit. (6) erythraline hydriodide, m.p. 252–253° dec.]. The IR spectra were also superimposable.

A portion of the aqueous layer left after removal of Fraction B was acidified with dilute sulfuric acid to pH 1.0. It was refluxed for 3 hr., made alkaline with dilute ammonia solution, and extracted with chloroform (5 × 200 ml.). The yield of the residue, after complete solvent removal, was 0.8 g. (Fraction C). It was chromatographed over alumina, but no pure base or salt could be isolated.

The other portion of the aqueous layer was acidified and precipitated with Dragendorff's reagent. A part of the separated precipitate (35 g.) was decomposed with moist silver carbonate (15 g.) and processed to obtain a crystalline material. It was recrystallized from ethanol to give a base, m.p. 250° dec. [lit. (18) hypaphorine, m.p. 253–254°].

Anal.—Calcd. for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37. Found: C, 67.81; H, 7.11; N, 11.10. The base showed no depression on determining mixed melting point with authentic hypaphorine, and the IR spectra of both were identical.

The base formed a hydrochloride, m.p. 231–232° dec. [lit. (19) hypaphorine hydrochloride, m.p. 234–235°].

Anal.—Calcd. for $C_{14}H_{18}N_2O_2 \cdot HCl$: C, 59.48; H, 6.77; Cl, 12.54; N, 9.91. Found: C, 59.10; H, 6.63; Cl, 12.42; N, 9.80.

The hydrobromide, m.p. 233° dec. [lit. (20) hypaphorine hydrobromide, m.p. 225°], and nitrate, m.p. 221–222° dec. [lit. (18) hypaphorine nitrate, m.p. 223.5–224.5°], of the base were also prepared.

Mandi Seeds—The seed powder (4.8 kg.) was extracted with petroleum ether (60–80°) and the extract was worked as described previously to give an alkaloidal entity (2.5 g., Fraction D). The marc was extracted with ethanol, and the extract was processed to give alkaloidal Fraction E (14 g.).

A benzene-soluble portion (11 g.) of Fraction E was resolved on an alumina column (330 g.). Elution with benzene first gave a syrupy mass (3.0 g.) and then a solid residue (1.5 g.) which crystallized from acetone to give a base, m.p. 208–210° [lit. (13) erysodine, m.p. 204–205°].

Anal.—Calcd. for $C_{18}H_{21}NO_3$: C, 72.22; H, 7.07; N, 4.68. Found: C, 72.34; H, 6.95; N, 4.77. $[\alpha]_D^{20}$ + 220.8° (c, 0.98, $CHCl_3$) [lit. (13) $[\alpha]_D^{27}$ + 248° (c, 0.311, EtOH)], UV $\lambda_{max.}^{MeOH}$ 285 mμ (log ε 3.6) [lit. (17) 285 mμ (log ε 3.6)]. The base showed no depression on determining mixed melting point with authentic erysodine. A mixture with the latter was inseparable on TLC, and the IR spectra were identical.

The hydrochloride was prepared by treating absolute ethanolic solution of the base with ethanolic hydrochloric acid. It was crystallized from dry ether–absolute ethanol to give an entity, m.p. 217–218° dec.

Anal.—Calcd. for $C_{18}H_{21}NO_3 \cdot HCl$: C, 64.40; H, 6.61; Cl, 10.56; N, 4.17. Found: C, 64.66; H, 6.68; Cl, 11.02; N, 4.30.

The syrupy mass was picked up with dry ether and made slightly acidic with ethanolic hydrochloric acid. The separated mass was repeatedly crystallized from dry ether–absolute ethanol to give a product, m.p. 206–208° [lit. (21) erysotrine hydrochloride, m.p. 205–206°].

Anal.—Calcd. for $C_{19}H_{23}NO_3 \cdot HCl$: C, 65.22; H, 6.91; Cl, 10.14; N, 4.01. Found: C, 65.34; H, 7.26; Cl, 10.30; N, 4.02. UV $\lambda_{max.}^{EtOH}$ 232 mμ (ε 19,800) and 284 mμ (ε 3490).

A part of the aqueous layer left after separation of alkaloidal Fraction E was hydrolyzed and worked as for Fraction C to obtain 2.1 g. (Fraction F) of the "liberated" base. The latter was resolved over alumina (53 g.). Elution with chloroform yielded a solid residue (1.1 g.), which crystallized from acetone to give a base, m.p. 208–210°, identified as erysodine.

Anal.—Calcd. for $C_{18}H_{21}NO_3$: C, 72.22; H, 7.07; N, 4.68. Found: C, 72.46; H, 7.04; N, 4.90.

The aqueous layer left after the removal of the "free" (Fraction E) and "liberated" (Fraction F) bases gave hypaphorine when worked in the usual way.

Fraction D (2.5 g.) was chromatographed over alumina (75 g.). Elution with benzene gave a syrupy mass (1.25 g.), which gave an alkaloidal hydrochloride, m.p. 203–204°, identified as erysotrine hydrochloride.

Anal.—Calcd. for $C_{19}H_{23}NO_3 \cdot HCl$: C, 65.22; H, 6.91; N, 4.01. Found: C, 65.24; H, 6.93; N, 3.90.

Joginder Nagar Seeds—This collection, when worked in the same manner as Mandi seeds, led also to the isolation of erysodine and erysotrine, the latter obtained as the hydrochloride.

The isolated bases and salts were identified by comparison with authentic specimens.[5]

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NMR Analysis of Some Alkyl *p*-Hydroxybenzoates

F. SHIHAB, W. SHEFFIELD, J. SPROWLS, and J. NEMATOLLAHI*

Abstract □ By applying NMR spectrometry, a number of solutions containing unknown quantities of two or three alkyl *p*-hydroxybenzoates (parabens) in 60% polyethylene glycol in water were analyzed for quantification of the individual parabens. The method was found to be accurate, facile, and rapid and seems to possess a promising potential for applicability in related fields of pharmaceutical analysis.

Keyphrases □ Parabens—NMR analysis □ Alkyl *p*-hydroxybenzoates—NMR analysis □ NMR spectroscopy—analysis

The antibacterial and antifungal properties of esters of *p*-hydroxybenzoic acid (parabens) have been of interest to pharmaceutical scientists for the past few decades. Methyl, propyl, butyl, and benzyl parabens have been investigated individually or in combination in pharmaceutical research and development.

A literature survey revealed that UV spectrophotometry is the most widely used method for a quantitative analysis of the parabens (1). This technique has proved quite satisfactory in the quantification of a single paraben, but it is not sufficient for the analysis of a mixture of parabens in solution. This is due to the fact that most parabens possess nearly identical $\lambda_{max.}$ values.

Procedures such as column chromatography (2) and GLC (3) have been reported for the analysis of parabens. The former procedure is time consuming and laborious. If esters are in an aqueous solution, the silylation technique, which should precede injection onto the column, together with other problems inherent to GLC makes the latter procedure less desirable and casts some doubts upon the accuracy of the results.

An exploratory attempt on the applicability of NMR spectrometry for the analysis of parabens revealed the method to be both rapid and accurate and the procedure quite simple.

EXPERIMENTAL

The parabens used in the experiments were all reagent grade. The general procedure for preparing the standard solutions consisted of placing an accurately weighed quantity of the desired ester in a volumetric flask, dissolving, and adjusting the volume with 60% polyethylene glycol (PEG) 400 in water. This vehicle was also used for preparing the solution of the paraben mixtures. Adoption of this solvent was due to its routine use in some other aspects of the research and to illustrate the point that the analysis does not require deuterated solvents.

The NMR spectra were determined using a Varian A-60 NMR spectrophotometer at an ambient temperature.

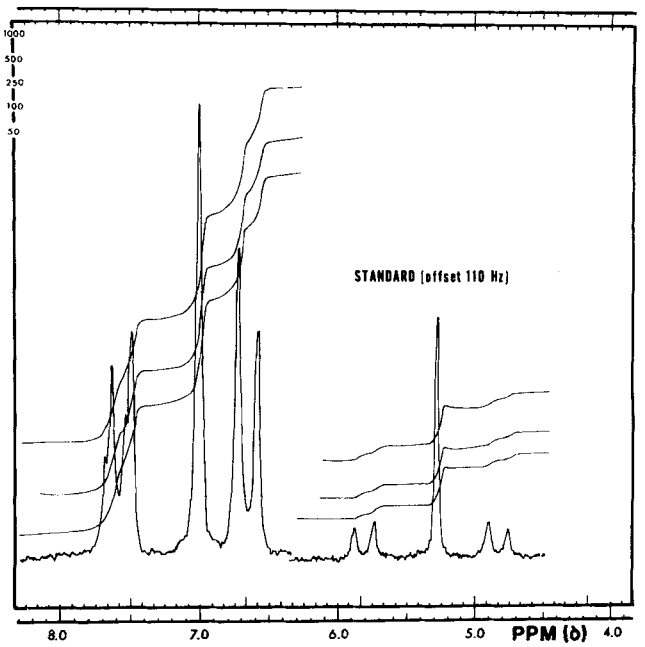

Figure 1—*The NMR spectrum of a mixture of benzyl paraben and methyl paraben in PEG–H_2O. Standard benzyl paraben is depicted at 110 c.p.s. offset.*

RESULTS AND DISCUSSION

Depicted in Fig. 1 is the NMR spectrum of a mixture of benzyl paraben and methyl paraben in 60% PEG in water. Benzyl paraben contains two doublets at δ 6.85 and δ 7.85, which are the results of spin-spin coupling of two pairs of phenyl protons, in two different magnetic environments, in the *p*-hydroxybenzoate moiety of the ester molecule (Structure I). The singlet at δ 7.3 results from the phenyl protons of the benzyl moiety. The two doublets of benzyl paraben superimpose on the doublets of methyl paraben.