Phytochemistry, 1977, Vol. 16, pp. 1427–1429. Pergamon Press. Printed in England.

ROTUNDIFOLINE *N*-OXIDES

E. J. SHELLARD, P. J. HOUGHTON and P. K. LALA

Pharmacognosy Research Laboratories, Department of Pharmacy, Chelsea College, University of London, Manresa Road, London, SW3 6LX, U.K.

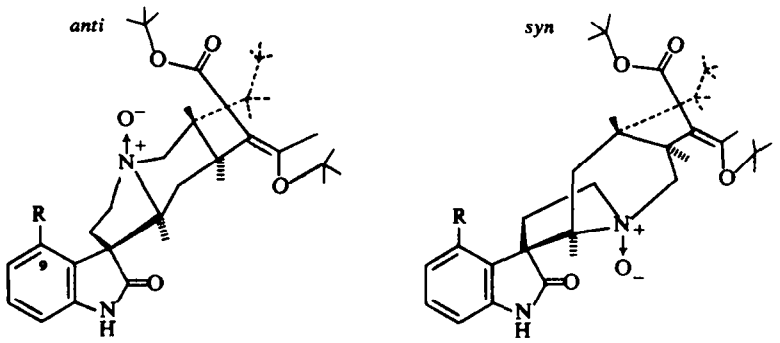
(*Revised received* 17 *March* 1977)

Key Word Index—*Mitragyna rubrostipulata*; Rubiaceae; *N*-oxides; oxindoles; alkaloids; rotundifoline.

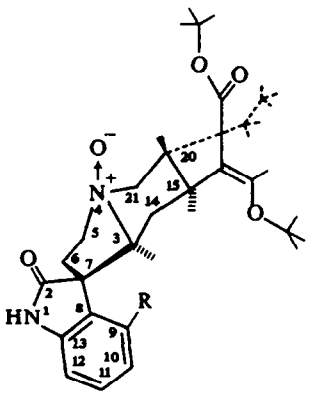
Abstract—The *N*-oxides of rotundifoline are prepared for comparison with a new alkaloid from *Mitragyna rubrostipulata*.

INTRODUCTION

Among the oxindole alkaloids isolated from species of *Mitragyna* are the C(9)-H, C(9)-OH and C(9)-OMe substituted *normal* open E ring alkaloids together with *N*-oxides of the C(9)-H and C(9)-OMe members of the group [1]. All three series of alkaloids exist in two isomeric forms, the A and B series based upon the configuration at the spiro carbon 7. The A series of oxindole alkaloids are those in which the lactam carbonyl of ring B lies below the plane of the C/D ring junction while in the



A oxindole *N*-oxides



B oxindole *N*-oxide
R = H, OH, OMe

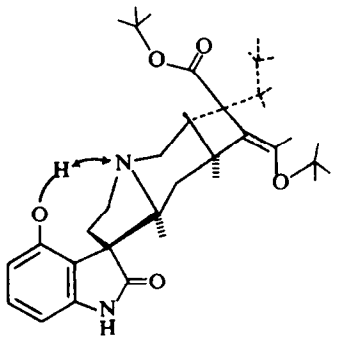
A oxindoles

anti isorhynchophylline *N*-oxide,	*syn* isorhynchophylline *N*-oxide
anti rotundifoline *N*-oxide	*syn* rotundifoline *N*-oxide
anti rhynchociline *N*-oxide	*syn* rhynchociline *N*-oxide

B oxindoles

rhynchophylline *N*-oxide
isorotundifoline *N*-oxide
ciliaphylline *N*-oxide

Scheme 1.



rotundifoline

Scheme 2.

B series, the lactam carbonyl lies above the plane of the C/D ring junction.

It has been shown that whereas the B series oxindoles give only one *N*-oxide the A series oxindoles give two—an *anti* and a *syn* *N*-oxide. The configuration of the three *N*-oxides of the C(9)-H and the C(9)-OMe *normal* open E ring oxindole alkaloids have been clearly elucidated [2, 3] (Scheme 1).

Rotundifoline and isorotundifoline are the A and B series C(9)-OH substituted alkaloids in the group respectively, but whereas the latter is a phenolic alkaloid, rotundifoline behaves as a non-phenolic alkaloid because of strong hydrogen bonding between the $N_{(4)}$ and the H of the hydroxy group [4] (Scheme 2). It was, therefore, considered that while isorotundifoline could be oxidized to give an *N*-oxide this would not be possible with rotundifoline. However, during the examination of leaves, stem bark and root bark of *Mitragyna rubrostipulata* (Schum) Havil, a polar oxindole alkaloid (considered to be a *N*-oxide because of its behaviour on TLC was isolated, which on reduction with H_2SO_3 yielded rotundifoline [5]. In order to confirm this it was necessary to prepare the *N*-oxide(s) of rotundifoline.

RESULTS AND DISCUSSION

Rotundifoline treated (a) with *m*-perchlorbenzoic acid and (b) with H_2O_2 yielded three products as shown by TLC, one of the products being present in extremely small quantities compared with the other two. They were separated by preparative TLC. The two major compounds have a UV and NMR spectrum identical with that of rotundifoline. Their MS are very similar, each one having the molecular ion peak at *m/e* 416 indicating the presence of an additional oxygen. Upon reduction with H_2SO_3 both compounds yielded rotundifoline.

The CD curves both showed positive Cotton Effect in the region 280–295 nm which confirmed that the *N*-oxides were those of rotundifoline and not isorotundifoline, thus substantiating the TLC evidence [6]. They are, therefore, considered to be stereoisomeric *N*-oxides of rotundifoline and were differentiated by examination of the MS fragmentation pattern. The percentage relative abundance of the molecular ion in one *N*-oxide was greater than in the other (13:8) thus indicating the former to be the *anti*-rotundifoline *N*-oxide and the latter to be the *syn*-rotundifoline *N*-oxide [7]. Further confirmation of this lies in the fact that the oxidation processes gave greater yields of the *anti*-rotundifoline *N*-oxide than of the *syn*-rotundifoline *N*-oxide: though no precise measurements were made it was approximately in the ratio 2.5:1 [3].

The oxidation of isorotundifoline with H_2O_2 gave only one oxidation product which on reduction with H_2SO_3 yielded isorotundifoline only as shown by TLC. MS gave molecular-ion peak *m/e* 416 but no further spectra were obtained. The *N*-oxides of rotundifoline were thus given the structures as shown in Scheme 1, where R = OH.

The naturally occurring *N*-oxide corresponded to the *anti*-rotundifoline *N*-oxide and this corresponds to the configuration of the *anti*-isorhynchophylline *N*-oxide in *Mitragyna inermis* [2] and to the *anti*-rhynchociline *N*-oxide in *Mitragyna tubulosa* [3] (Scheme 1).

Because of the very low quantity available no spectral work was undertaken on the oxidation product PKL3 except the MS which gave *m/e* 413.

EXPERIMENTAL

The 90 MHz NMR spectra were determined in $CDCl_3$ using TMS as internal reference; MS were determined at 70 eV with inlet temp. 180°. The TLC systems used were Silica gel G (Merck) with (a) $CHCl_3$–Me_2CO (5:4); (b) $CHCl_3$–EtOH (6:1); (c) EtOAc–*iso*-PrOH–5.5% NH_4OH (14:5:1); (d) MeOH. The hR_f values are as follows: Rotundifoline, (a) 73, (b) 90, (c) 85 (d) 74. Isorotundifoline, (a) 58, (b) 85 (c) 82, (d) 67; *anti*-rotundifoline *N*-oxide, (a) 0, (b) 30, (c) 25, (d) 57: *syn*-rotundifoline *N*-oxide, (a) 0, (b) 5, (c) 5, (d) 14: isorotundifoline *N*-oxide, (a) 0, (b) 29, (c) 21, (d) 25: PKL 3. (MW 414), (a) 0, (b) 14, (c) 14, (d) 46.

Preparation of alkaloid N-*oxides.* Method 1: The alkaloid was treated as previously described [2] the preparative TLC system being system (d). Rotundifoline (80 mg) yielded *anti*-rotundifoline *N*-oxide (15 mg), *syn*-rotundifoline *N*-oxide (6 mg) and traces of a 3rd oxidation product (PKL 3). Isorotundifoline (10 mg) yielded isorotundifoline *N*-oxide (1.3 mg). Method 2: The alkaloid was treated as previously described [2] and separately by preparative TLC as for Method 1.

Characterization of prepared N-*oxides.* The UV spectra (EtOH) of the *N*-oxides were similar to those of the corresponding tertiary bases: rotundifoline, λ_{max} 222, 242 *sh* 288–296; isorotundifoline, λ_{max} 220, 242 *sh* 286–295. The NMR data is given in Table 1. Reduction of 1 mg or less with 5% H_2SO_3 as previously described [2] yielded one spot on TLC having an R_f value identical with that of the corresponding tertiary base. The MS data are as follows: *anti*-rotundifoline *N*-oxide *m/e* 416 (M^+ 14%), 400 (M^+-16, 63%), 398 (43%), 239 (100%), 224 (97%), 210 (29%) 208 (38%), 175 (45%), 162 (22%), 161 (20%), 160 (47%) 146 (32%), 65 (100%), *syn*-rotundifoline *N*-oxide *m/e* 416 (M^+ 9%), 400 (M^+-16, 85%) 398 (19%), 239 (28%), 224 (89%) 210 (46%), 208 (59%), 175 (18%), 162 (13%), 161 (12%), 160 (26%), 146 (38%), 69 (100%). CD (MeOH) data—*anti*-rotundifoline *N*-oxide $[\theta]_{203} + 6.5 \times 10^{-3}$,

Table 1. NMR data of C(9)-OH normal oxindole *N*-oxides

Protons	*anti*-Rotundifoline *N*-oxide	*syn*-Rotundifoline *N*-oxide	Rotundifoline
C(19)-Me	0.80t	0.79t	0.80t
—OMe (ester)	3.62s	3.61s	3.60s
—OMe (vinyl)	3.72s	3.72s	3.70s
C(10)-H	6.80d	6.80d	6.35d
C(11)-H			7.00m
C(12)-H	7.10m	7.1 m	6.58d
C(17)-H	7.26s	7.25s	7.22s
NH	8.05s	8.0s	8.30s

$[\theta]_{232} + 7.1 \times 10^{-3}$, $[\theta]_{255} - 2.54 \times 10^{-3}$, $[\theta]_{292} + 0.40 \times 10^{-3}$: *syn*-rotundifoline *N*-oxide $[\theta]_{235} + 5.8 \times 10^{-3}$, $[\theta]_{259} - 1.24 \times 10^{-3}$, $[\theta]_{294} + 0.10 \times 10^{-3}$.

Characterization of the natural rotundifoline N-*oxide.* The naturally occurring rotundifoline-*N*-oxide obtained from the total alkaloidal extract of *Mitragyna rubrostipulata* by column and preparative TLC was shown by means of the UV spectrum (EtOH), the NMR spectrum, the CD spectrum, the MS and the hR_f values to be identical with the prepared *anti*-rotundifoline *N*-oxide.

Acknowledgements—We thank Mr. D. Carter, School of Pharmacy (University of London) Brunswick Square, London, for determination of the MS, Dr. P. M. Scopes, Chemistry Department, Westfield College (University of London) Hampstead, London, and Mr. C. McDonough of this College for NMR spectra. One of us (PKL) thanks the Vice Chancellor of the Jadavpur University for extended leave of absence, thus enabling him to undertake research work in the Pharmacognosy Laboratories, Department of Pharmacy, Chelsea College.

REFERENCES

1. Shellard, E. J. (1974) *Bull. Narcotics* **26**, 41 and cited references.
2. Shellard, E. J., Phillipson, J. D. and Sarpong, K. (1971) *Phytochemistry* **10**, 2505.
3. Phillipson, J. D., Rungsiyakul, D. and Shellard, E. J. (1973) *Phytochemistry* **12**, 2043.
4. Beckett, A. H. and Tackie, A. N. (1964) *Chem. Ind.* 1122.
5. Shellard, E. J., Houghton, P. J. and Lala, P. K. (1977) *J. Pharm. Pharmac.* in press.
6. Trager, W. F., Lee, C. M., Phillipson, J. D., Haddock, R. E., Dwuma-Badu, D. and Beckett, A. H. (1968) *Tetrahedron* **24**, 523.
7. Phillipson, J. D. and Hemingway, S. R. (1973) *Phytochemistry* **12**, 2791.