that the red fruits of *Rivinia humilis* L. contain, besides betanin and isobetanin, a red–violet pigment (rivinianin). In the present study the rivinianin has been shown to be betanin 3′-sulphate.

Rivinianin was isolated from aqueous extracts of fruits by preparative electrophoresis. Since the pigment (λ_{max} 253 541 nm; mobility relative to betanin 1·78 at pH 2·4 and 1·34 at pH 4·5) gave on complete acid hydrolysis a mixture of betanidin and isobetanidin, it is a betanidin derivative; the hydrolysate also contained glucose identified by PC (six solvents) and sulphate identified via $BaCl_2$. Alkaline hydrolysis of rivinianin in the absence of oxygen gave, besides sulfuric acid, a mixture of betanin and isobetanin identified by paper electophoresis, analytical column chromatography on polyamide and treatment with β-glucosidase which gave glucose and a mixture of betanidin and isobetanidin [3,4]. Since diazomethane methylation of rivinianin followed by alkali fusion gave 5-hydroxy-6-methoxyindole-2-carboxylic acid, it was inferred that the hydroxyl group at position 6 of the aglycone is free. In order to ascertain the position of the SO_3^- group in the glucose residue, rivinianin was treated with MeI in $HCONMe_2$ in the presence of AgO. The permethylated product on acid hydrolysis gave 2,4,6-tri-*O*-methyl-D-glucose; from this it follows that only one SO_3^- group is present in the molecule and this is linked to the hydroxyl group at position 3′ of the glucose residue.

Acknowledgement—The author thanks Prof. M. Piattelli for his interest and criticism.

REFERENCES

1. Wyler, H., Rosler, H., Mercier, M. and Dreiding, A. S. (1967) *Helv. Chim. Acta* **50**, 545.
2. Piattelli, M., and Minale, L. (1964) *Phytochemistry* **5**, 547.
3. Minale, L., Piattelli, M., De Stefano, S. and Nicolaus, R. A. (1966) *Phytochemistry* **5**, 1037.
4. Minale, L., Piattelli, M. and De Stefano, S. (1967) *Phytochemistry* **6**, 703.

Phytochemistry, 1975, Vol. 14, pp. 2527–2529. Pergamon Press. Printed in England.

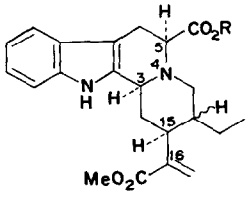
ADINA ALKALOIDS: ISOLATION AND STRUCTURE OF ANHYDROADIRUBINE

RICHARD T. BROWN and ANTONIOS A. CHARALAMBIDES
Department of Chemistry, The University, Manchester M13 9Pl England

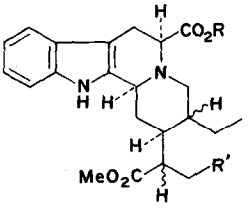
(*Received* 21 *February* 1975)

Key Word Index—*Adina rubescens*; Rubiaceae; carboxyindole alkaloid; anhydroadirubine.

The Malaysian tree *Adina rubescens* has yielded several indole alkaloids with novel structural features. In particular, a unique group containing a carboxyl function constituted the first representatives of a hitherto unknown series with standard monoterpenoid C_{10} units but derived directly from tryptophan rather than the ubiquitous tryptamine [1–3]. We now report the discovery of a second tetracyclic carboxy *Corynanthe* alkaloid —anhydroadirubine, for which structure **1a** is suggested.

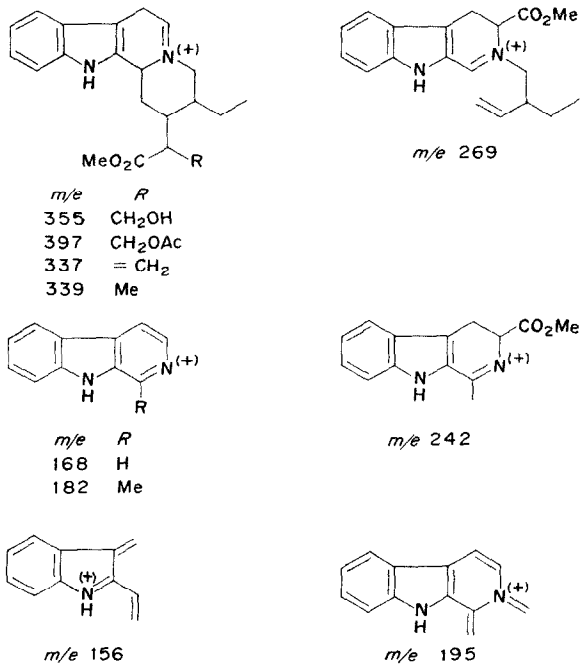


(1a) R = H
(1b) R = Me



	R	R′
(2a)	H	OH
(2b)	Me	OH
(2c)	Me	OAc
(2d)	Me	H

Chromatography of a methanolic extract of the heartwood from *A. rubescens* on ion exchange resins, followed by gel permeation afforded an amino-acid concentrate. After methylation and preparative TLC a small amount of an amorphous alkaloid $C_{23}H_{28}O_4N_2$ $[\alpha]_D^{25}$ −28° was obtained. Its UV spectrum indicated an indolic chromophore, confirmed by NMR signals for four aromatic protons in the τ 2·5–3·1 region and an indolic NH at τ 2·18, with a corresponding IR band at 3480 cm^{-1}. Two IR carbonyl absorptions at 1745 and 1720 cm^{-1} and a pair of methoxyl singlets in the NMR spectrum at τ 6·20 and 6·23 suggested two methyl esters, one of which was



Scheme 1.

possibly conjugated with an olefinic methylene group evident from an IR peak at 1630 cm^{-1} and two one-proton singlets at τ 3·70 and 4·35. A distorted 3-proton triplet at τ 9·12 established an ethyl group.

Dominant mass spectral fragments (see Scheme 1) at *m/e* 337 (M-CO_2Me), 335 (M-CO_2Me-2H), 242 and 168 were characteristic of a tetrahydro-β-carboline substituted at C-5 by a carbomethoxy group, and immediately reminiscent of methyl adirubine (**2b** [1]. The resemblance extended to substantial M-1, 195, 182 and 156 ions consistent with a fourth ring linked to C-3 and N-4 in the unknown alkaloid also. However, one major difference was the lack of any substantial ion derived by cleavage of the 15-16 bond and loss of the "C_3" unit which was so apparent with methyl adirubine, e.g. *m/e* 269.

Confirmation of the olefinic bond was obtained by catalytic hydrogenation to a mixture of two isomeric dihydro-derivatives. In addition to the above features the mass spectra now exhibited ions at *m/e* 269 (M-87) etc., attributable to a 15–16 cleavage and loss of MeO_2C-CH-Me. From this behaviour it could be deduced that the original compound must have contained a conjugated ester moiety MeO_2C-C^{16} = CH_2 where (i) cleavage of the bond adjacent to the sp [2] hybridized C-16 would not be favoured, and (ii) hydrogenation would generate a new chiral centre at C-16, and thus two products. At this stage the most probably gross structure of the alkaloid was that of methyl anhydroadirubine (**1b**). Since the CD spectrum showed a positive Cotton effect between 250 and 300 nm, the absolute configuration at C-3 must be α (S), and *trans*-quinolizidine IR bands in the 2700–2800 cm^{-1} region indicated that H-3 was *cis* to H-15 which thus had the expected α configuration.

In view of this stereochemical correspondence at two centres, a chemical correlation with adirubine seemed feasible. Accordingly, elimination of acetic acid from methyl adirubine acetate (**2c**) with refluxing methanolic sodium methoxide gave methyl anhydroadirubine (**1b**) identical with the isolated alkaloid by IR, NMR, CD spectra and TLC in several systems. Further, since methyl adirubine was recovered unchanged from the reaction, no epimerisation of H-5 had occurred, indicating that the 5-carbomethoxy group was already in the more stable equatorial orientation and hence β. This means that H-5 in adirubine and its derivatives have the α (S) configuration in agreement with a derivation from L-tryptophan. Hence the isolated methyl ester is represented by structure **1b**, the naturally occurring carboxy alkaloid presumably being anhydroadirubine (**1a**) by analogy with adirubine (**2a**).

EXPERIMENTAL

Isolation of methyl anhydroadirubine (**1b**). Powdered heartwood (1 kg) of *Adina rubescens* was extracted with MeOH (8 l) which was evaporated under red pres to give an orange powder (100 g). Methanolic sol was passed down an Amberlyst 15 (H^+) column and elution with MeOH–Et_3N afforded a basic concentrate (22 g) which was transferred to an Amberlyst A26 (OH^-) column. Elution with MeOH–AcOH gave an amino-acid concentrate (16 g) which was chromatographed in two batches on Sephadex LH20 gel (600 g), eluting with MeOH and monitoring fractions (20 ml) by UV and TLC assay. Fractions 41–54 were combined, methylated with CH_2N_2 and separated by preparative TLC on Sil with $CHCl_3$–EtOAc (2:1) to give methyl anhydroadirubine (4 mg) as an amorphous powder $[\alpha]_D^{25}$ −28° ($CHCl_3$); M^+ 396·2058, $C_{23}H_{28}O_4N_2$ requires 396·2049; UV (MeOH): λ_{max} 289, 282, 273, 225 nm; IR ($CHCl_3$): ν_{max} 3480 (NH), 1745 (C=O), 1720 (C=O), 1630 (C=C) cm^{-1}; CD (MeOH): $[\theta]_{294_{max}}$ +4·3 × 10^3, $[\theta]_{289_{min}}$ +3·1 × 10^3, $[\theta]_{273_{max}}$ +6·2 × 10^3 deg. cm^2 $decimol^{-1}$; NMR ($CDCl_3$): τ 2·18 (*s*, NH), 2·5–3·1 (*m*, 9-12-H), 3·70 (*s*, 17-H_a), 4·35 (*s*, 17-H_b), 6·20 (*s*, CO_2Me) 6·23 (*s*, CO_2Me), 9·12 (*t*, 18-Me); MS: *m/e* 396, 395, 381, 367, 365, 338, 336, 335, 309, 242, 183, 182, 169, 168, 156.

Preparation of methyl 17-desoxyadirubine (2d). Methyl anhydroadirubine (1 mg) in MeOH (2 ml) was hydrogenated over Pd black (1 mg) for 3 hr. Filtration and evaporation afforded the 2 epimers of methyl desoxyadirubine; UV (MeOH): λ_{max} 289, 282, 273, 225 nm; MS, *m/e* 398 (M^+), 397, 340, 339, 337, 311, 309, 269, 251, 242, 183, 182, 169, 168, 156. TLC on Sil with $CHCl_3$–EtOAc (2:1) showed 2 overlapping spots.

Preparation of methyl anhydroadirubine. Methyl adirubine acetate (2c) (10 mg), prepared by methylation and acetylation of adirubine, was heated under reflux with NaOMe in dry MeOH for 45 min. After addition of a small lump of solid CO_2, solvent was removed under red pres the residue taken up in $CHCl_3$ and separated by TLC on silica with $CHCl_3$–EtOAc (2:1). In addition to methyl adirubine (2·6 mg), methyl anhydroadirubine (3·5 mg) was obtained and proved to be identical with the previously isolated material by MS, IR, NMR, CD spectra and TLC in several systems.

Acknowledgement—We thank the S.R.C. for financial support of this work and a post-doctoral fellowship (A.A.C.).

REFERENCES

1. Brown, R. T., Chapple, C. L. and Lee, G. K. (1972) *Chem. Commun.* 1007.
2. Brown, R. T. and Charalambides, A. A. (1974) *Tetrahedron Letters* 1649, 3429.
3. Brown, R. T. and Chapple, C. L. (1973) *J.C.S. Chem. Commun.* 886.

Phytochemistry, 1975, Vol. 14, p. 2529. Pergamon Press. Printed in England.

CALYCANTHINE FROM *PALICOUREA ALPINA*

REX B. WOO-MING and KENNETH L. STUART
Department of Chemistry, University of the West Indies, Kingston 7, Jamaica

(*Received* 21 *April* 1975)

Key Word Index—*Palicourea alpina*; Rubiaceae; alkaloid; calycanthine.

Plant. Palicourea alpina (Sw.) DC. *Source.* Newcastle, St. Andrew, Jamaica, collected in October 1970 (Herbarium No. 27,179, Botany Department, University of the West Indies). *Previous work.* Plant material from Hardwar Gap, St. Andrew collected in July 1973 was shown to contain vomifoliol[1], indole alkaloids palinine, palidimine and harman[2].

Present work. Stems and leaves were dried and powdered (150 g). Extraction with 2% tartaric acid followed by the usual work up for basic material[1] yielded 0·275 g of crude material. PLC on silica plates using $CHCl_3$–MeOH (1:1) afforded 12 mg of a chromatographically homogeneous solid which had identical physical properties (UV, IR[3], NMR[4] to calycanthine. High resolution MS showed that the base peak was the parent ion (*m/e* 346·2113; Calculated for $C_{22}H_{26}N_4$, 346·2157) and the fragmentation pattern observed was very similar to that reported earlier for calycanthine[5].

Calycanthine has previously been shown to be the principal poisonous constituent of *Calycanthus glaucus* Willd.[6] and was also reported from *C. floridus* L.[7] and *C. occidentalis* Hook. and Arn.[8] (Calycanthaceae). *Chimonanthus praecox* (L.) Link (= *Meratia praecox* Rehder and Wilson) Calycanthaceae[9] and *Bhesa archboldiana* (Merr. and Perry) Ding Hou (Celastraceae)[10] have also been shown to contain this alkaloid, but this is the first report of its isolation from the Rubiaceae. It is noteworthy that specimens of *Palicourea alpina* growing at Hardwar Gap, only 2 km from the location of the plants used in this study, did not yield this alkaloid, but other indole types were shown to be present[2].

Acknowledgements—We thank Professor J. P. Kutney and Dr. Guenter Eigendorf, Department of Chemistry, Univeristy of British Columbia, for high resolution MS data. One of us (R.B.W.) acknowledges his indebtedness to the Government of Guyana for study leave during which this work was carried out.

REFERENCES

1. Stuart, K. L. and Woo-Ming, R. B. (1975) *Phytochemistry* **14**, 594.
2. Stuart, K. L. and Woo-Ming, R. B. (1974) *Tetrahedron Letters* 3853.
3. *Physical Data of Indole and Dihydroindole Alkaloids*, Vol. 1, 1961, Lilly Research Laboratories, Eli Lilly, Indiana, U.S.A.
4. Woodward, R. B., Yang, N. C., Katz, T. J., Clark, V. M., Harley-Mason, J, Ingleby, R. F. J and Sheppard, N. (1960) *Proc. Chem. Soc.* 76.
5. Clayton, E., Reed, R. I., and Wilson, J. M. (1962) *Tetrahedron* **18**, 1495.
6. Eccles, G. R. (1888) *Proc. Am. Pharm. Assoc.* **84**, 382.
7. Späth, E. and Stroh, W. (1925) *Ber. Deut. Chem. Ges.* **58**, 2131.
8. Manske, R. H. F. and Marion, L. (1939) *Can. J. Res.* B17, 293.
9. Manske R. H. F. (1929) *J. Am. Chem. Soc.* **51**, 1836.
10. Culvenor, C. C. J., Johns, S. R., Lamberton, J. A. and Smith, L. W. (1970) *Aust. J. Chem.* **23**, 1279.