Tetrahedron, 1967, Vol. 23, pp. 3129 to 3145. Pergamon Press Ltd. Printed in Northern Ireland

INDOLE ALKALOIDS FROM GAMBIR STRUCTURE OF GAMBIRTANNINE, OXOGAMBIRTANNINE AND DIHYDROGAMBIRTANNINE*

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(*Received* 4 *October* 1966; *accepted for publication* 24 *November* 1966)

Abstract—On the basis of chemical and spectral evidence, the structures I, II and III were assigned to gambirtannine, dihydrogambirtannine and oxogambirtannine, three new indole alkaloids extracted from the tannin Gambir. The phenol betaine IV (neooxygambirtannine) was obtained during the workup of the extract.

GAMBIR (or Gambier) is a tanning material, produced by evaporation of the aqueous extract of leaves and stems of the Rubiacea *Uncaria gambier* Roxb. (*Ourouparia gambir* Baillon), a tree growing in South-East Asia.

In 1934 Raymond-Hamet[1] reported the presence in *Uncaria gambier* of an alkaloid with sympatholytic activity, named gambirine, without giving details of the extraction or chemical data. Some years later Pavolini *et al.*[2] described the extraction from the tannin of a fluorescent light-sensitive alkaloid with the probable formula $C_{22}H_{26}N_2O_4$, for which they proposed again the name gambirine. Raymond-Hamet claimed later[3] that the two substances were different. In 1962 Spiteller *et al.*[4] extracted 11-methoxyyohimbine from *Aspidosperma oblongum* A. DC. and suggested it was identical with Pavolini's gambirine, on the basis of the m.p. and analytical data.[5] We wish to report here the results of our investigation on the tannin.

Extraction of the tannin with 30% NaOH and ether gave a crude basic fraction exhibiting a strong yellow-green fluorescence. The mixture must be immediately purified, because it is light- and air-sensitive. Rapid chromatography through neutral alumina afforded four compounds, *gambirtannine* (I), *dihydrogambirtannine* (II), *oxogambirtannine* (III) and *neooxygambirtannine* (IV). The total yield in these products

* Presented to the IUPAC International Symposium on the Chemistry of Natural Products, Stockholm 1966.

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[1] Raymond-Hamet, *Bull. Acad. Méd. Paris* [3] **112**, 513 (1934).

[2] T. Pavolini, F. Gambarin and G. Montecchio, *Ann. Chim. Rome* **40**, 654 (1956).

[3] Raymond-Hamet, *C.R. Acad. Sci., Paris* **245**, 1458 (1957).

[4] G. Spiteller and M. Spiteller-Friedmann, *Monatsh.* **93**, 795 (1962).

[5] Quoted also by J. Poisson, *Ann. Chim. Paris* **9**, 99 (1964) as stating the "doubtless" identity with gambirine.

is about 0·05%. The first substance is the main source of the fluorescence and has properties similar to those given by Pavolini.[2,*]

Gambirtannine decomposes, even in the solid state, on standing in the air, giving rise to a small amount of oxogambirtannine. A solution of gambirtannine in many solvents rapidly gives a mixture, from which I, III and IV can be recovered. It should be noted that IV does not appear in the crude extract, and thus it is an artifact.

Gambirtannine analyses well for $C_{21}H_{18}N_2O_2$ (confirmed by high-resolution mass spectroscopy) and is optically inactive. The UV spectrum shows max at 266 (sh), 314, 340, 410 mμ (ε = 10500, 10840, 12800, 22400) in 95% EtOH, and at 250 (sh) and 358 mμ in acidified EtOH (ε = 11300, 23000), whereas the IR spectrum (KBr) shows bands at 3·0 (NH), 5·87 (conjugated ester), 6·15–6·30 (C=C and aromatic), and 12–14 μ (aromatic).

Oxogambirtannine (III) analyses for $C_{21}H_{16}N_2O_3$, has no optical activity, and UV max at 256, 300, 346 (sh), 368, 385 mμ (ε = 13410, 5300, 17200, 25900, 23650) in 95% EtOH. The IR spectrum shows a complex absorption near 3·0, again the conjugated ester band at 5·83, a strong peak at 6·08 (amide CO) and unsaturation bands at 6·2–6·4 μ.

The analytical data, together with a positive (green) Adamkiewicz reaction, suggested that these two compounds belong to the class of the so-called indole alkaloids; however, they possess an unusually low number of hydrogen atoms.[6] The long wave-length absorptions in the UV spectra and the fluorescence were in agreement with strongly conjugated systems. This was confirmed by an explorative catalytic hydrogenation of gambirtannine, from which a product with a typical unsubstituted indole cromophore was obtained, as shown later.

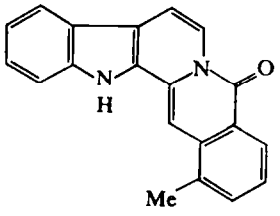
In order to obtain information on the skeleton structure, selenium dehydrogenation of I and III was undertaken. A strongly yellow fluorescent product was obtained in low yield from gambirtannine. It exhibited absorption max in the UV at 290, 300, 320, 335, 370 (sh), 389, 412, 440 (sh) mμ. After one day the absorption pattern of the alcoholic solution appeared completely different, with maxima at 289 and 380 mμ. This behaviour has been reported[7] to be typical of *dehydroketoyobirine* (V). Direct comparison of the UV spectrum and TLC behaviour with those of a sample of dehydroketoyobirine prepared by selenium dehydrogenation of yohimbine confirmed that the compounds were identical. The mass spectrum of the product from gambirtannine showed, however, in addition to dehydroketoyobirine (*m/e* 298), the presence of about 30% of a second compound with a molecular ion peak at 284. This substance was also the main product of the Se dehydrogenation of oxogambirtannine (together with a small amount of a compound with *m/e* 342), and has UV and IR (3·0 μ, NH; 6·08 μ, amide CO) spectra very similar to those of V. It must then be *nordehydroketoyobirine* (VI). The formation of both compounds in the dehydrogenation of gambirtannine is easily explained, by assuming that the ready conversion of I into III can have occurred in part before the dehydrogenation.

Gambirtannine and oxogambirtannine have then a yohimbine skeleton. Independent evidence came from the alkaline hydrolysis of oxogambirtannine to the

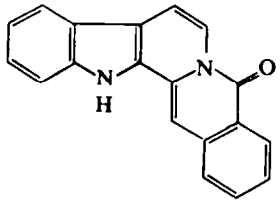
* We prefer to adopt the name "gambirine" to indicate one of the alkaloids present in the plant.

[6] M. Hesse, *Indolalkaloide in Tabellen*. Springer-Verlag, Berlin (1964).

[7] A. LeHir, R. Goutarel, M. M. Janot, and A. Hofmann, *Helv. Chim. Acta* 37, 2161 (1954).

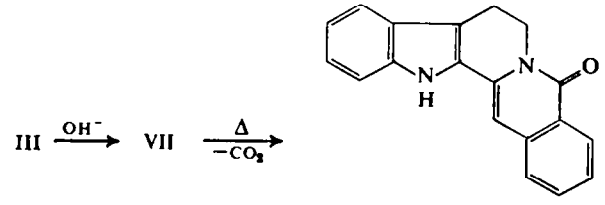


V



VI

corresponding acid VII. Pyrolysis of the latter afforded a blue fluorescent substance, which, when isolated by preparative TLC, exhibited the same UV spectrum as norketoyobirine[8] (VIII):



VIII

Oxogambirtannine should be therefore, a norketoyobirine bearing a carbomethoxy group.

The NMR spectrum of oxogambirtannine (III), reported in Fig. 1, shows two symmetrical triplets of two protons centered at 3·08 and 4·49 δ, attributed to the sequence $\gtrless$—CH_2(6)—CH_2(5)—N$\lesssim$. This pattern has to be described as a deceptively simple[9] ABXY* type, where $\delta_{AB} = 0$, and $\delta_{XY} = 0$, and the geminal couplings J_{AB} and J_{XY} are large compared with the difference between *cis* and *trans* couplings, i.e. $\frac{1}{2}(J_{AX} - J_{AY})$ and $\frac{1}{2}(J_{BX} - J_{BY})$ are small.[10]

In this cyclic molecule there is no possibility of free rotation around the —C(5)—C(6)— bond to average the *cis* and *trans* couplings (A_2X_2 type spectrum); hence the separation of 6·5 c/s between the lines in each triplet will be equal to $\frac{1}{2}(J_{AX} + J_{AY} + J_{BX} + J_{BY})$. The strong downfield shift of the two protons near $N_{(b)}$ (4·49 δ) is explained by the partial positive charge localized on the amide nitrogen, and the deshielding effect of the C=O in the *peri* position (both the protons lie out of the positive conical region of the C=O, and, taking into account the flipping at C-5, they lie

* The symbols adopted are those used by J. W. Emsley, J. Feeney and L. H. Sutcliffe, *High Resolution Nuclear Magnetic Resonance Spectroscopy*. Pergamon Press, Oxford (1965).

[8] G. A. Swan, *J. Chem. Soc.* 486 (1949). The numbering of the curves in Fig. 1, page 489, of this paper is apparently reversed.

[9] The conditions to obtain a simple ABXY of two triplets are given by R. J. Abraham and H. J. Bernstein, *Canad. J. Chem.* **39**, 216 (1961).

[10] Here the rapid interconversion of ring C at room temperature must occur, in order to explain the two pairs of symmetrical triplets in the spectrum of such a non-symmetrical molecule. Due to the flipping of C-5 (and resp. C-6) the chemical shifts are equal ($\delta_A = \delta_B$ and $\delta_X = \delta_Y$) and the difference between the *cis* and *trans* coupling constants decreases.

approximately in the plane of the C=O). This effect is lacking in the gambirtannine (I) spectrum, where the same ≈—CH_2—CH_2—N≺ sequence gives a narrow deceptively simple ABCD pattern centered at 3·10 δ.

In addition, in the spectrum of III appear signals of 1 OMe (3·82 δ), 1 NH (9·13 δ), a singlet (1 H) at 7·99 δ, and two signals each of 1 H, with both *ortho* and *meta* splittings. The latter are the AM part of a AMX spectrum: H_X, dd, 8·30 δ, J_{AX} =

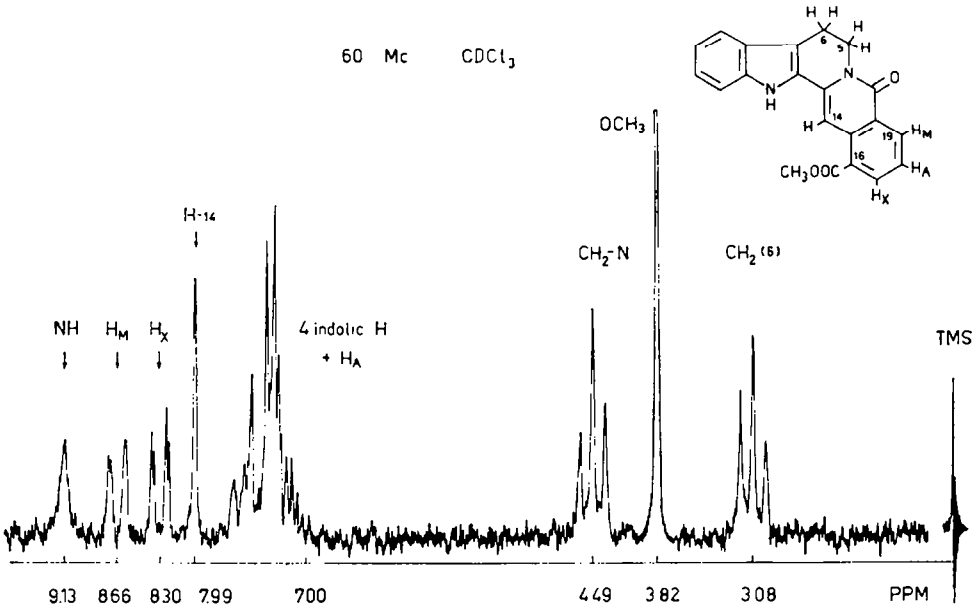


FIG. 1

7·5, J_{MX} = 1·5; H_M, dd, 8·66 δ, J_{AM} = 8·0, J_{MX} = 1·5, plus another interaction of less than 1 c/s. H_A is submerged in the multiplet (total 5 H) of the indolic protons spread between 7·0 and 7·8 δ.

The spectrum of I ($CDCl_3$) shows, besides the above mentioned ABCD pattern (≈—CH_2—CH_2—N≺) at 3·10 δ, one OCH_3 (3·89 δ), one indolic NH signal (8·55 δ), a singlet of two H at 4·19 δ (N—CH_2-aryl).[11] The aromatic region absorption differs from that of III: only a doublet of doublets is visible at 7·82 with splittings of 7·0 and 2·0 c/s (X part of an ABX spectrum), the other protons being superimposed on the complex absorption (7 H) spread between 6·7 and 7·7.

Now the reaction gambirtannine (I) → oxogambirtannine (III) can be interpreted as the oxydation of the group >N—CH_2—C≪ to a lactam >N—CO—C≪, as is apparent from the stoichiometry of the reaction and the amide absorption (similar to that of ketoyobirine) in the IR spectrum of III. That the oxydation takes place at C-21 is proved by the disappearance of the CH_2 absorption at 4·19 δ in the NMR spectrum of III, and the appearance of the two protons on C-5 at low field in III and

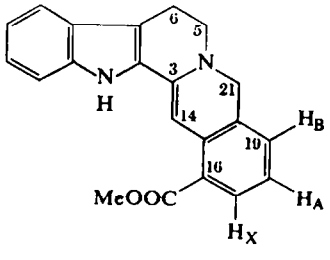
[11] The same chemical shift for these two protons, leading to a one-line AB pattern, may be explained by the flipping of $N_{(b)}$ or by a slight distortion of C-21 in the half-chair ring D, in such a way as to give the two protons the same relationship with respect to the aromatic ring and to the nitrogen lone pair. The increased planarity of the conjugated system and the decreased interaction at C-5 and C-21 bonds could contribute to the stability of this latter conformation.

not in I, as discussed above. Such an oxydation of a 1,2-dihydroisoquinoline to isocarbostyril is well known, and a closely related example is the conversion of alstoniline into alstoniline oxide.[12]

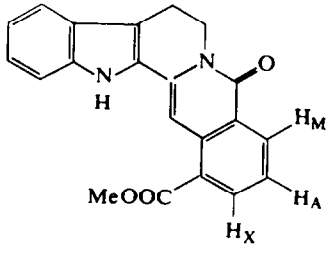
The placing of the carbomethoxy group on C-16 although apparent from biogenetic reasons, is established by the results of the selenium dehydrogenation and by some features of the aromatic pattern in the NMR spectrum of III. From the coupling constants and line intensities of H_X and H_M the three protons must be *ortho* to each other. The strong deshielding of H_M (8·66 δ) is due to the carbonyl in the *peri* position, and H_X is also deshielded (8·30 δ) by the *ortho* effect of the carbomethoxy group; this excludes the possibility of placing the carbomethoxyl on C-19. Comparing these values with those of the same protons in gambirtannine, the strong upfield shift of H_{19} (=H_B) is evident, from 8·66 δ to values $<$7·60 δ; it has now about the same shift as H_A, both being overlapped by the other aromatic protons. Also H_X and H_{14} move upfield (8·30 → 7·82 δ; 7·99 → $<$7·60 δ) because of the lack of the carbonyl in the *para* position.

The formation of V from I during selenium dehydrogenation implies a rearrangement typical of the yohimbine derivatives,[13] whereas the dehydrogenation of III, which already has an amide CO at C-21, proceeds only through the loss of the carbomethoxy group. The peak at *m/e* 342 in the mass spectrum of the dehydrogenation product of III can then be assigned to the molecular peak of a small amount of 5,6-dehydrooxogambirtannine. Moreover, additional evidence that the COOMe is on C-16 comes from the upfield shift of H-14 (6·35 δ) when the carbomethoxyl is reduced to CH_2OH (compound XI) with respect to gambirtannine ($<$6·90 δ).

On the basis of all the preceding data, we can write the following formulae I and III for gambirtannine and oxogambirtannine respectively:



I



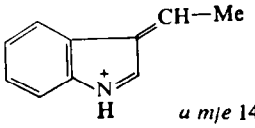
III

The second alkaloid eluted during the chromatography of the crude extract, II, $C_{21}H_{20}N_2O_2$ (MS), m.p. 163°, $[\alpha]_D^{20} = -270°$ ($CHCl_3$, $c = 0{\cdot}088$), gives a yellow-brown fluorescence in UV light, a blue positive Adamkiewicz reaction, and shows a typical unsubstituted indole cromophore (λ_{max} 225, 283, 290 mμ, $\varepsilon = 60900$, 12750, 11500 in 95% EtOH). The IR exhibits bands at 3·03 (indole NH), 3·5–3·7 (Bohlmann bands), 5·90 μ (unsaturated ester) and those characteristic of aromatic absorption.

The mass spectrum of compound II ($M^+ = 332$) is shown in Fig. 2. A fragment

[12] [a] R. C. Elderfield and J. L. White, *J. Org. Chem.* **19**, 683 (1954); [b] R. C. Elderfield and O. L. McCurdy, *Ibid.* **21**, 295 (1956).

[13] E. W. Warnhoff, in P. De Mayo, *Molecular Rearrangements*, p. 890. Interscience, New York (1964).



a m/e 144

ion peak at *m/e* 144 (*a*, $C_{10}H_{10}N_1$)[14] is characteristic for the alkaloids of this type.[15] A retro Diels-Alder reaction in ring D gives fragment *b* (*m/e* 162, $C_{10}H_{10}O_2$). The corresponding two-nitrogen-containing fragment seems to be generated by a different process. If the charge is on the $N_{(b)}$ atom the hydrogen at C-3 can be lost, which leads to the strong $M^+ - 1$ peak (*m/e* 331, $C_{21}H_{19}N_2O_2$); or the C(3)—C(14) bond can break, with the formation of *c*. In *c* the C-14 radical can attack a hydrogen at C-5, which gives *d*, and after a charge transfer from $N_{(b)}$ to $N_{(a)}$ and through the loss of the

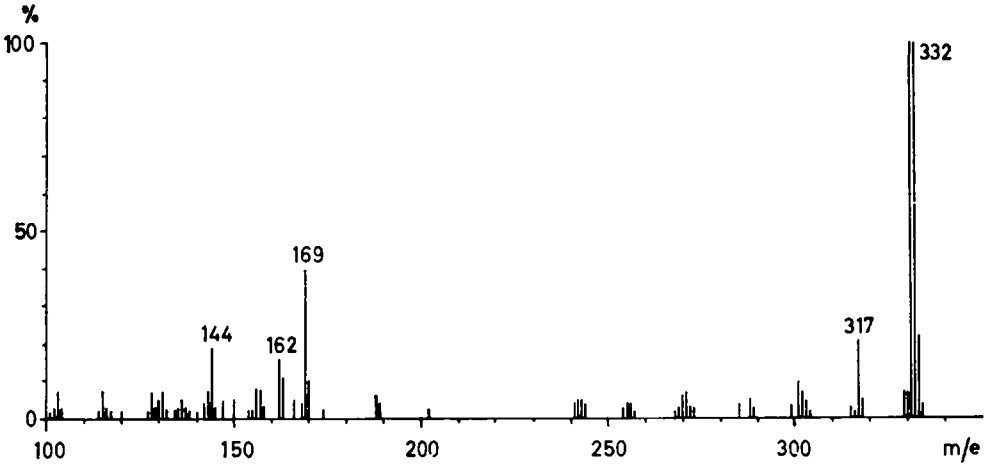


FIG. 2

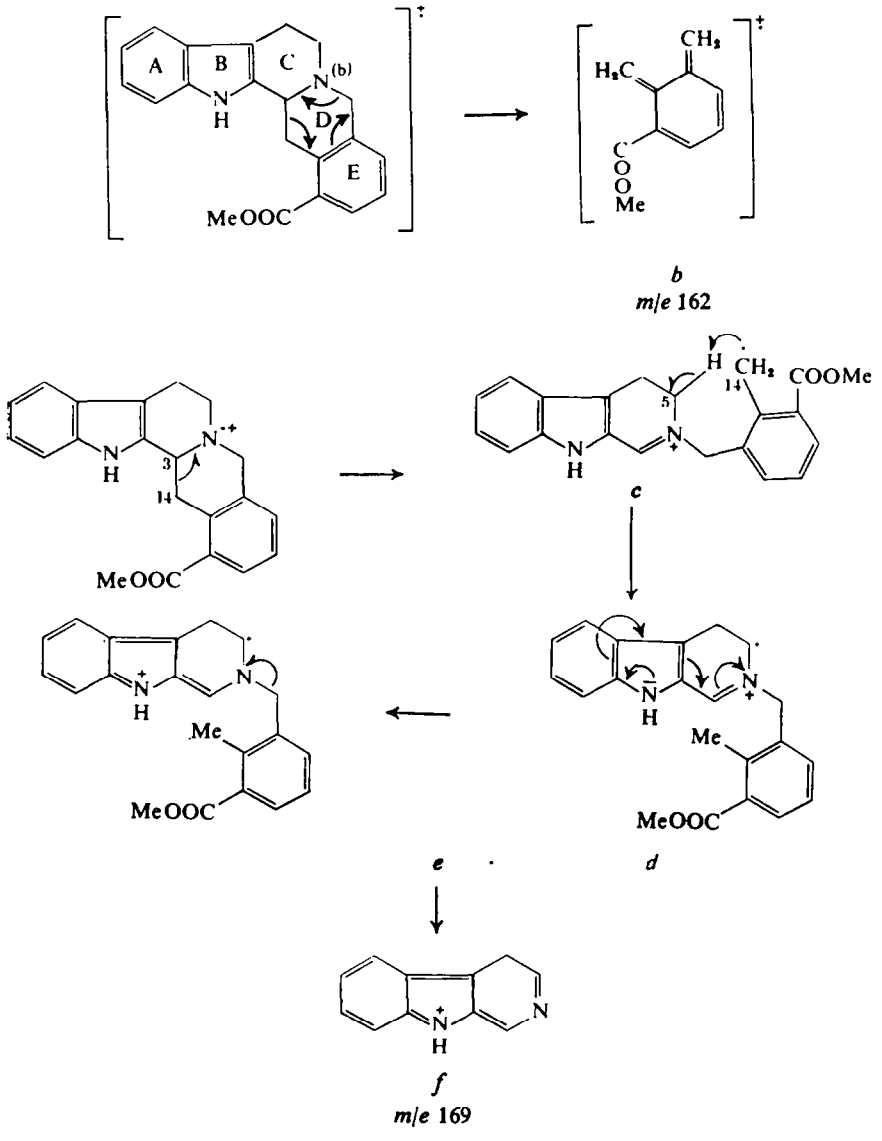
aromatic $N_{(b)}$-substituent, ion *f*, (*m/e* 169, $C_{11}H_9N_2$) is formed.[16] This fragmentation proves that the indolic nucleus is not substituted, that ring D is saturated, and that ring E is aromatic and bears the carbomethoxy group.

The 60 Mc NMR spectrum ($CDCl_3$) of II shows the same indole type absorption between 6·7 and 7·6 δ (integrated value of 6 H) as compound I, a triplet of 1 H at 7·84 δ, and the indolic NH signal at 8·10 δ. At 100 Mc (Fig. 3) the signal at 7·84 δ is a quartet with splittings of 3·5 and 5·5 c/s, which collapses to a singlet after 71 c/s upfield irradiation. This means that it is the X part of an ABX spectrum with a small chemical shift difference (δ_{AB}); H_A and H_B are overlapped by the 4 indolic protons. Considering $J_{AX} = 7{\cdot}5$–$8{\cdot}0$ c/s and $J_{BX} = 1{\cdot}5$ c/s (as for *ortho* and *meta* couplings in alkaloid III) the value $J_{AX} + J_{BX} = 9{\cdot}0$–$9{\cdot}5$ c/s fits well with the separations between transitions 9 and 12 (= 9 c/s). These features suggest the presence of the aromatic ring E with the same substitution as in gambirtannine (—COOMe at 16 and lack of

[14] All elementary compositions of ions were obtained by high resolution mass spectroscopy.

[15] cf. H. Budzikiewicz, C. Djerassi and D. H. Williams, *Structure Elucidation of Natural Products by Mass Spectroscopy*, Vol. I; *Alkaloids* p. 41. Holden-Day (1964).

[16] A very similar fragmentation pattern was also shown by the known compound XII, which differs from II only for the presence of a primary alcohol function, instead of a carbomethoxyl, in position 16.

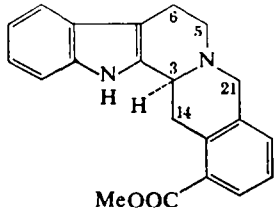


C=O at 21). The complex absorption at upper field measures on integration 12 H: it includes the OMe (3·86 δ), the ABXY type pattern[17] of the 4 H at C-5 and C-6, the ABC pattern of >N—CH(3)—CH_2(14)-aryl and 2 protons at C-21. The latter do not experience the same chemical shift, the equatorial one being visible at lower field (4·10 δ), as a doublet with J_{gem} = 15·5 c/s. Evidence that this is a coupling constant is proved by no change of the separation at 60 and 100 Mc or with different solvents ($CDCl_3$, acetone, benzene). The axial proton should be shielded by the nitrogen lone pair in a *trans* relationship.[18] To this alkaloid, *dihydrogambirtannine*, belongs then the

[17] The lack of symmetry of such a pattern (cf. oxogambirtannine) may be due to a more rigid conformation of ring C.

[18] F. Bohlmann, D. Schumann and H. Schulz, *Tetrahedron Letters* 173 (1965).

structure II:



II

which also represents the absolute configuration at carbon 3. The absolute configuration is established by the recent preparation[19] of the enantiomer of II, with $[\alpha]_D^{25} = +288°$ (pyridine) by degradation of deserpideine, which has the 3β configuration. Then dihydrogambirtannine must have the 3α one. The presence of the Bohlmann

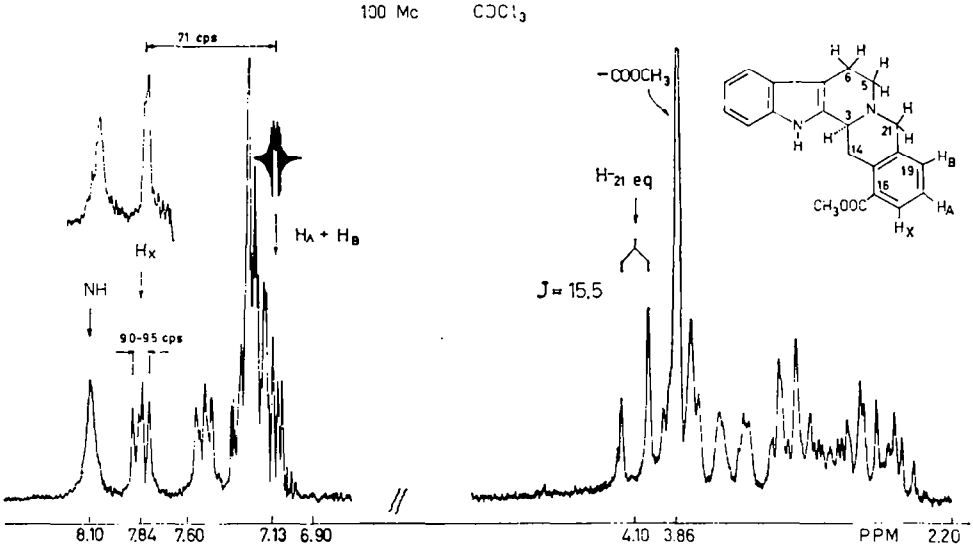


FIG. 3

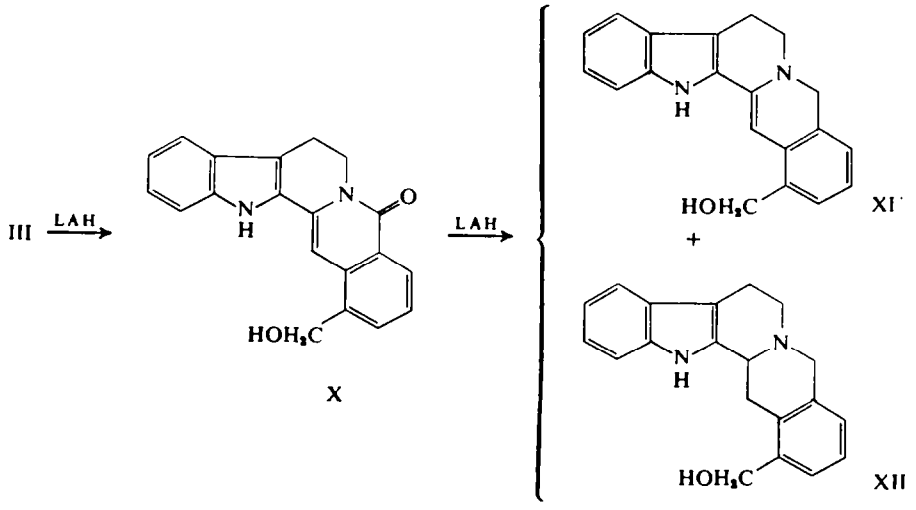
bands[20] at 3·50, 3·57 and 3·62 μ in the IR spectrum ($CHCl_3$) of II, and the lack of any signal due to H_3 at a field lower than 4·0 δ[21] in the NMR spectrum, clearly indicate that H_3 is *trans diaxial* to the nitrogen lone pair, and thus the C/D rings are *trans* fused. The corresponding racemic compound IX was obtained by catalytic hydrogenation of gambirtannine. The UV, IR, NMR and mass spectra of IX are identical with the corresponding spectra of II.

[19] E. Smith, R. S. Jaret, M. Shamma and R. J. Shine, *J. Am. Chem. Soc.* **86**, 2083 (1964).

[20] E. L. Eliel, N. L. Allinger, S. J. Angyal and G. A. Morrison, *Conformational Analysis* p. 332. Interscience, New York (1965). See also W. F. Trager, C. M. Lee and A. H. Beckett, *Tetrahedron* **23**, 365 (1967).

[21] M. Uskokovic, H. Bruderer, C. von Planta, T. Williams and A. Brossi, *J. Am. Chem. Soc.* **86**, 3364 (1964) and literature quoted.

Oxogambirtannine (III) is strongly resistent to catalytic hydrogenation. Treatment with LAH for a short time led to the carbinol X:



With excess LAH a mixture of XI and XII was obtained, however always accompanied by some unreduced X. X and XII could not be separated even by TLC, so that pure XII[22] was prepared only by catalytic hydrogenation of purified XI, or by LAH reduction of IX.[19] The reactions could be easily followed with TLC, because of the strong fluorescence of the substances in UV light. X, XI and XII have UV absorptions similar to those of oxogambirtannine, gambirtannine, and indole, respectively. The mass spectra are consistent with the structures given. If the reaction mixture from LAH reduction of III is exposed to air for some time, red products similar to that obtained by chromatography of the crude extract of the tannin are formed.

Neooxygambirtannine, the fourth substance obtained from the chromatography of the tannin extract, red with red fluorescence in UV light, shows a strongly enhanced polarity on TLC and is apparently an oxidation product of gambirtannine. IV is an isomer of oxogambirtannine, with the same formula $C_{21}H_{16}N_2O_3$. The mass spectrum shows no characteristic fragmentation pattern: the most intense peak is that of the molecular ion, and loss of Me, OH and $C_2H_2O_2$ from M^+ are responsible for the fragment ion peaks. This result would have indicated an aromatic compound, whereas the UV spectrum (Fig. 4) has a long wavelength absorption pattern, which suggests a more delocalized structure. Selenium dehydrogenation gave again dehydroketoyobirine (V) or the norderivative (VI), thus indicating that the gambirtannine skeleton was not altered.

The NMR spectrum at 60 Mc ($CDCl_3$) shows the same simple ABXY pattern of two triplets found in oxogambirtannine, also with identical chemical shifts $\delta_A = \delta_B = 4{\cdot}49$ and $\delta_X = \delta_Y = 3{\cdot}09$. Consequently, the fragment >N—CH_2(5)—CH_2(6)— has to be similar, with an amide type nitrogen, or with a positive charge localized on it. Furthermore the spectrum shows a —COOMe (4·09 δ) and a complex aromatic absorption between 6·6 and 8·8 δ (8 H). At 100 Mc (DMSO-d_6, Fig. 5) in the aromatic

[22] R. C. Elderfield and B. A. Fischer, *J. Org. Chem.* **23**, 949 (1958). Prof. Elderfield kindly communicated to us that he had no more of it for comparison.

region of the spectrum, the singlet of H-21, strongly deshielded (8·32 δ) by the charged vicinal nitrogen, and the quartet of H-17 (7·95 δ) with *ortho* and *meta* splittings ($J = 1{\cdot}5$ and $J = 7{\cdot}8$ c/s) become visible. H_{18} and H_{19} lie together in the multiplet centered at 6·98 δ) as can be deduced from the line intensities of the H_{17} signal. The four indolic protons are responsible for the complex absorption between 7·20–7·70 δ. The presence of a proton giving a sharp signal at 11.32 δ, which does not exchange

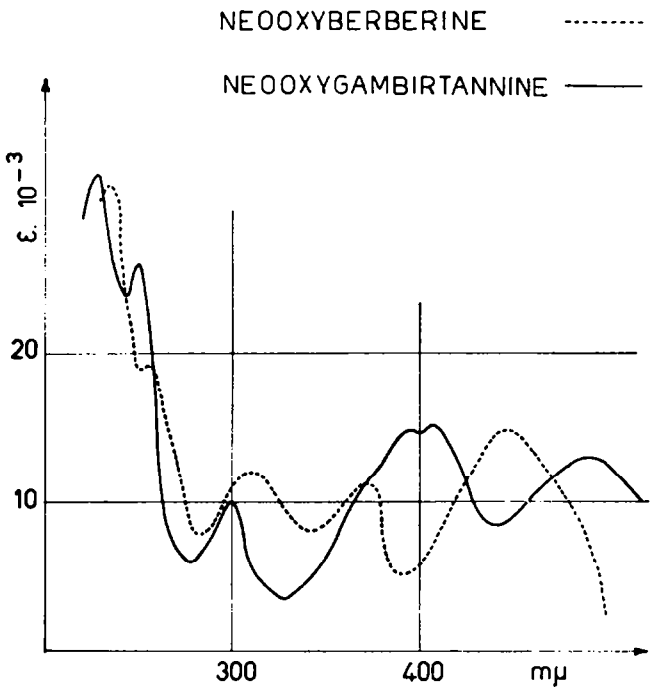
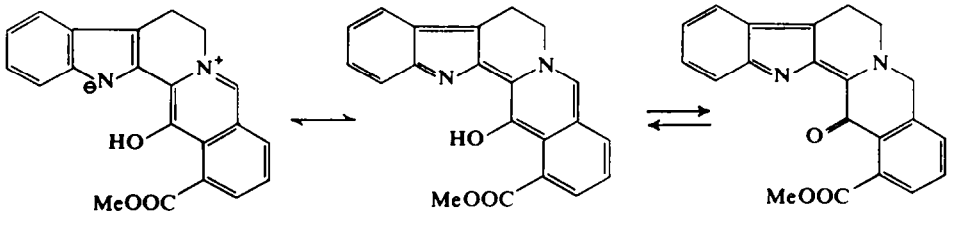


FIG. 4

with excess CF_3COOH[23] provides evidence for an indolic NH and rules out the structures IVa, b, c, in this solvent[24]:



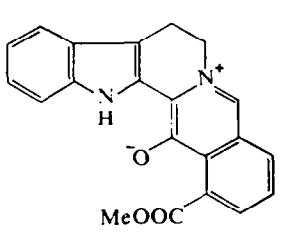
IVa IVb IVc

On the basis of these data, we propose for neooxygambirtannine the structure IV,

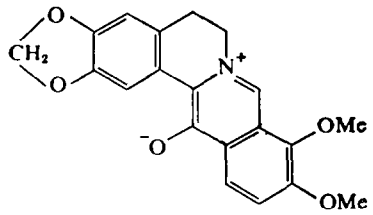
[23] After acidification the signal is still sharp at 11·59 δ, while the CF_3COOH signal is at 12·40 δ.

[24] Non-polar structures IVb and c cannot of course be excluded for the compound in the gas phase, that means under the mass spectrum recording conditions.

where oxidation has occurred at C-14:



IV



XIII

At this point the similarity between the chemistry of our compounds and that of berberine alkaloids should be noted. Particularly, $KMnO_4$ oxidation of acetone-berberine gives the phenol betaine *neooxyberberine* XIII[25], the UV spectrum of which is reported in Fig. 4 together with the spectrum of IV.

To confirm the structure IV, we have carried out the $NaBH_4$ reduction of neo-oxygambirtannine, as was recently used by Takemoto *et al.*[25] to reduce neooxy-berberine to D,L-ophiocarpine. Our reduction proceeded along a similar path, leading

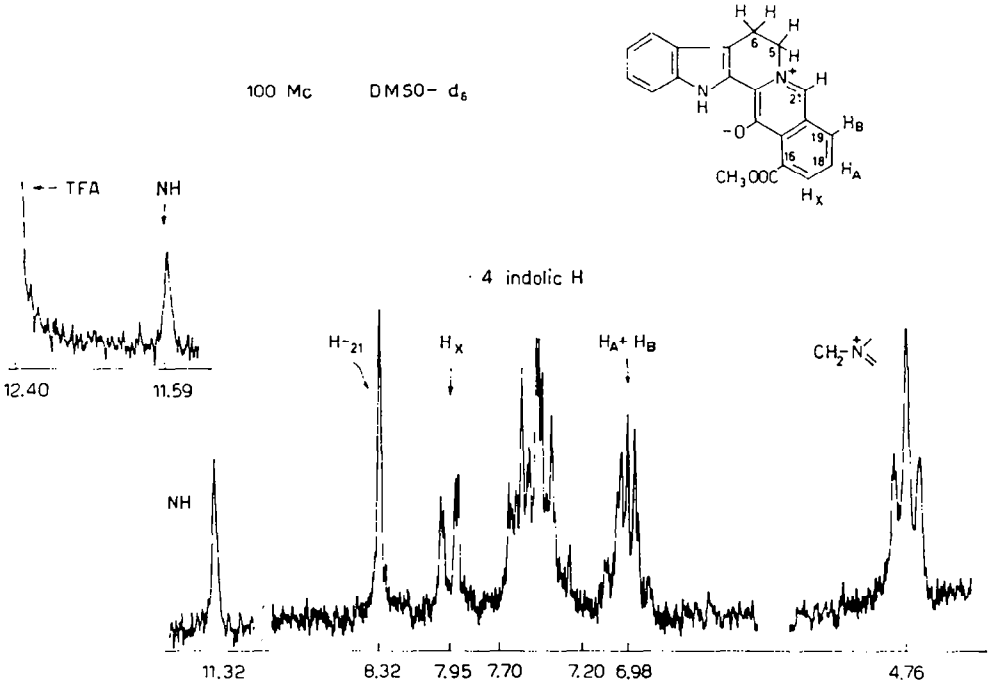
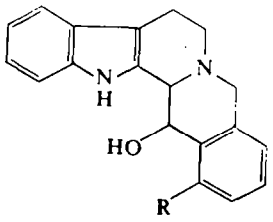


FIG. 5

to a product with an unsubstituted indole cromophore. Mass and IR spectra indicated that also a reduction of the carbomethoxy group has occurred, and that the product

[25] J. Pyman, *J. Chem. Soc.* **99**, 1690 (1911); C. Schöpf and M. Schweickert, *Chem. Ber.* **98**, 2566 (1965).
[26] T. Takemoto, Y. Kondo and K. Kondo, *Yakugaku Zasshi* **83**, 166 (1963); *Chem. Abstr.* **59**, 3970 (1963).

was a mixture of XIV and XV:

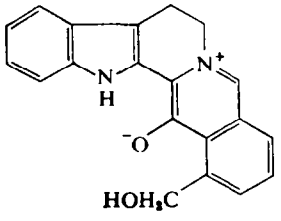


XIV R = COOMe

XV R = CH_2OH

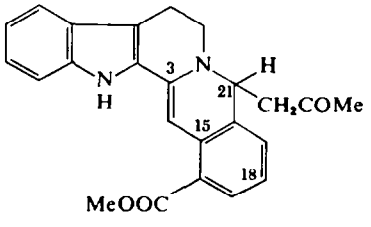
Moreover, the mass spectrum also shows the presence of a small amount of the compound I, gambirtannine, which is not, however, detectable by TLC. It is reasonable to assume that it is formed in the spectrometer by thermal dehydration, and that it must come only from XIV, thus confirming the structure of the later.

Two other red products with structures similar to that of neooxygambirtannine, XVI and XVIII, were obtained. The first one was formed, most probably by oxidation of XI, by leaving the reaction mixture from LAH reduction of III (see above)[27] in the air:

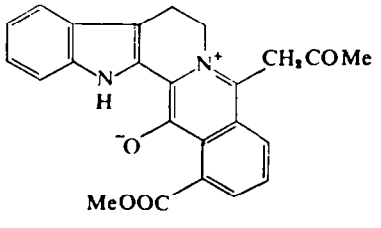


XVI

Reduction of XVI with $NaBH_4$ went further than in the case of IV, and gave only the carbinol XII, identified with MS and TLC. The other red compound, XVIII, was obtained from the acetone adduct of gambirtannine, XVII, which is easily prepared by chromatography of the crude extract of the tannin on alumina, when a hexane-acetone mixture is used as eluent.



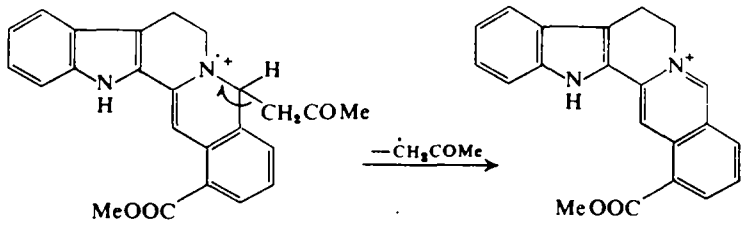
XVII



XVIII

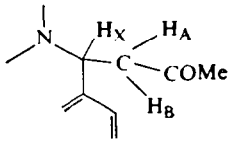
The attack of acetone at C-21 in compound XVII is clearly proved by its UV spectrum, similar to that of gambirtannine, and by NMR and mass spectra. The mass spectrum shows only a very small molecular peak at *m/e* 386 but a very intense peak at *m/e* 329 ($m^* = 280{\cdot}5$).

[27] The formation of red products, although not characterized from alstoniline under similar conditions has been reported.[12a]



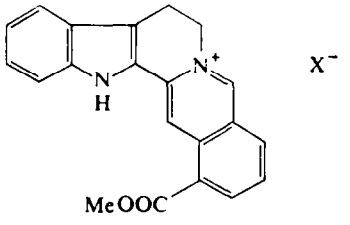
M⁺ 386 *m/e* 329

In the NMR spectrum of XVII ($CDCl_3$, 100 Mc), evidence for the group is given by the —COMe absorption at 1·87 δ and by the presence of an ABX pattern, where H_X is the proton at C-21 because of its low field shift (4·98 δ) and the lack of any additional



spin-coupling. The first order analysis gives: $\delta_A = 2{\cdot}97$, $\delta_B = 2{\cdot}64$, $\delta_X = 4{\cdot}98$; $J_{AB} = 16{\cdot}0$, $J_{AX} = 5{\cdot}5$, $J_{BX} = 6{\cdot}5$ c/s. Confirmation comes from the irradiation of H_X, which leads to an AB quartet with a geminal coupling of 16·0 c/s. Superimposed on the AB pattern is the methylene (C-6) absorption (2·70–3·14 δ); the CH_2(5) absorption is centered at 3·42 δ. The other parts of the spectrum are similar to that of gambirtannine.

Because of the new, strongly unsaturated structure of the compounds present in the tannin, we thought that a search for them in the plant *Uncaria gambier* would be interesting, in order to establish whether they were present in the plant, or were formed by influence of the production conditions (e.g. concentration at high temperature) from a more saturated precursor. In the meantime, and after the present work was already accomplished, a paper by Taylor and Raymond-Hamet[28] appeared, where they report the isolation of *ourouparine* (XIX) from *Uncaria gambier*, together with a small amount of oxogambirtannine (III). If ourouparine is present in the tannin, it might well give rise[29] to both I and III, during the alkaline treatment necessary for



the extraction. We have, however, obtained a batch of leaves and stems of *Uncaria gambier* from the Botanical Gardens of Singapore,[30] in which no fluorescent base was

[28] W. I. Taylor and Raymond-Hamet, *C.R. Acad. Sci., Paris* **262D**, 1141 (1966).

[29] W. J. Gensler, *The Chemistry of Heterocyclic Compounds* (Edited by R. C. Elderfield) Vol. 4; page 391, Wiley, New York (1952).

[30] Through the courtesy of Dr. A. G. Kenyon, Tropical Products Institute, London, whom we are glad to thank here again for his so kind cooperation.

found. Investigation on the thus far uncharacterized indole alkaloids of this plant is in progress.

EXPERIMENTAL

NMR spectra were measured with A-60 and HA-100 Varian spectrometers. Spin decoupling experiments were performed with the "frequency sweep" method. The integrals were measured with a 405/CR Hewlett-Packard digital voltmeter. Chemical shifts are in ppm (δ) from TMS, used as the internal standard. In the text, s = singlet, d = doublet, t = triplet, q = quartet. IR spectra of KBr disks were recorded with a Perkin–Elmer Infracord (values in μ), UV spectra of solutions in 95% EtOH were taken with a Beckman DK-2 apparatus. Mass spectra were measured with an AEI mass spectrometer type MS 9 (direct inlet system, 70 eV). M.ps were measured with a Kofler apparatus. The silica gel for column chromatographies was Merck 0·05–0·20 mm, for TLC it was Merck G.

Extraction of the tannin

Gambir cubes (purchased from Ledoga, Milano, 600 g)[31] were powdered and treated with 750 ml 30% NaOHaq, with stirring, until the brown mass became fluid. This had to be done as rapidly as possible, and with cooling in ice, in order to avoid the hydrolysis of gambirtannine. Ether (2 l.) were added and the mixture shaken 20 min in a brown bottle. The ether was decanted, and the extraction repeated until the extract was no longer fluorescent under UV light (Wood). The residue from evaporation of the solvent under reduced press was dissolved in $CHCl_3$ and immediately chromatographed through a 3 × 50 cm column filled with Al_2O_3 Woelm II. Elution with hexane–AcOEt gave 3 main fractions: A, with dark yellow fluorescence, (hexane–AcOEt 14:1), B, yellow fluorescence (10:1) and C, blue-green fluorescence in UV light (4:1). A last red fraction D was eluted only with AcOEt–MeOH 98:2. Evapn. of A and crystn. from hexane–ether gave 80 mg of *gambirtannine* (I), orange needles, m.p. 150–153° dec. (Found: C, 76·46; H, 5·47; N, 8·60. $C_{21}H_{18}N_2O_3$ requires: C, 76·34; H, 5·49; N, 8·48%.) MS: *m/e* 330 (M^+, 72%), 329 (100%), 269 (7%), 164 (double charged ion, 6%), 134·5 (8%). High resolution data:

Found	Calc.	Composition
330·1352 ± 0·0016	330·1368	$C_{21}H_{18}N_2O_3$
329·1293 ± 0·0016	329·1290	$C_{21}H_{17}N_2O_3$

The residue from evapn. of B was washed with hexane to remove the chlorophyll present and crystd. from ether–hexane to give 40 mg of *dihydrogambirtannine* (II), soft yellow crystals, m.p. 163°, ORD in MeOH (*c*, 0·1138): $[\alpha]_{600} = -370$, $[\alpha]_{500} = -500$, $[\alpha]_{400} = -835$, $[\alpha]_{350} = -1100$, $[\alpha]_{330} = -1405$, $[\alpha]_{315} = -1605$.

MS: see Fig. 2. Elemental composition of the important peaks:

Found	Calc.	Composition
331·1459 ± 0·0016	331·1446	$C_{21}H_{19}N_2O_3$
169·0763 ± 0·0009	169·0766	$C_{11}H_9N_2$
162·0696 ± 0·0016	162·0681	$C_{10}H_{10}O_2$
144·0807 ± 0·0014	144·0813	$C_{10}H_{10}N$

Fraction C gave ca. 100 mg *oxogambirtannine* (III), bright yellow, yellow-green fluorescent, m.p. 205° ($CHCl_3$–hexane). (Found: C, 72·93; H, 5·04; N, 8·05. $C_{21}H_{16}N_2O_3$ requires: C, 73·24; H, 4·68; N, 8·14%.) MS: *m/e* 344 (M^+, 100%), 329 (11%), 311 (15%), 284 (15%), 255 (14%),

[31] In a sample of Gambier FG 212, kindly provided to us by Compagnie Francaise des Extraits, Maison Westphalen, Le Havre, fluorescent compounds were absent.

172 (M^{2+}, 8%), 142 (*m/e* 284$^+$, 6%), (double charged ion, 22%). High resolution data:

Found	Calc.	Composition
344·1168 ± 0·0017	344·1161	$C_{21}H_{16}N_2O_2$
329·0928 ± 0·0016	329·0926	$C_{20}H_{13}N_2O_2$
311·0824 ± 0·0016	311·0820	$C_{20}H_{11}N_2O_2$
284·0960 ± 0·0014	284·0950	$C_{19}H_{12}N_2O$
255·0924 ± 0·0013	255·0922	$C_{18}H_{11}N_2$

The fraction D usually had to be rechromatographed through silica gel with the same eluent as above, to give ca. 10 mg *neooxygambirtannine* (IV), red, m.p. 220°, UV: 227·5, 250, 299, 394, 403, 490 mμ (ε = 31,900, 26,100, 9940, 14,780, 15,200, 12,900). MS: *m/e* 344 (M^+, 100%), 329 (12%), 311 (11%), 286 (26%), 284 (24%), 271 (11%), 255 (17%), 128 (double charged ion, 25%).

When the crude extract was chromatographed with hexane-acetone, a new green fluorescent band appeared, which was intermediate between B and C. Evapn. and crystn. from hexane–acetone yielded 21-*acetonylgambirtannine* (XVII), yellow, m.p. 178° dec, UV: 314, 345, 415 mμ (ε = 12,450, 14,800, 21,900). MS: *m/e* 386 (M^+, 3%), 330 (55%), 329 (100%), 328 (38%), 327 (38%), 165·5 (15%), 165 (13%).

Selenium dehydrogenations

(a) Compound I (150 mg) and Se (160 mg) were heated 1 hr at 300°, cooled, extracted with ether, then repeatedly with hot MeOH. The residue from the green fluorescent MeOH soln. was mixed with silica gel, charged on the top of a silica gel column and eluted with hexane–AcOEt. A few mg of a product were obtained, identical by UV spectra and TLC behaviour with a sample of dehydroketoyobirine prepared from yohimbine.

(b) Compound III (100 mg) and Se (100 ml) were reacted and worked up as above. A few mg of *nordehydroketoyobirine* (VI) were obtained. UV: 222, 252, ~260, 292, 301, 322, 337, ~370, 392, 415, ~445 mμ (ε = 26,200, 15,300, ~14,100, 11,500, 11,700, 6150, 6150, ~7900, 12,900, 12,600, ~5400). MS: *m/e* 284 (M^+, 100%), 255 (21%), 227 (4%), 142 (M^{2+}, 8%), 128 (double charged ion, 13%), 114 (d.c.i., 7%). High resolution of ion *m/e* 284: Found 284·0958 ± 0·0014, calc. 284·0950, comp. $C_{19}H_{12}N_2O$.

(c) Exactly similar reactions on II and IV gave small amounts of a green-yellow fluorescent product, indistinguishable from dehydroketoyobirine by comparison of UV spectra and TLC behaviour.

(d) Yohimbine (4 g) were dehydrogenated with Se as described by Barger and Scholz.[33] The MeOH extract contained some dehydroketoyobirine, together with ketoyobirine, Further dehydrogenation with Pd black[34] afforded again a mixture of both compounds. Pure *dehydroketoyobirine* (V) (some mg) was obtained by preparative TLC with hexane–AcOEt 2:1.

Norketoyobirine (VIII). 20 mg III were dissolved in 6 ml 1N KOH in MeOH and refluxed 10 min. The soln, evapd, taken up with dil. AcOH, and extracted with $CHCl_3$, gave VII, blue fluorescent in UV, dec. > 280°, IR 5·90, UV: 254 sh, 350 sh, 364, 380 sh (ε = 12,800, 21,100, 24,300, 19,900). Pyrolysis of VII at 280–300° *in vacuo* yielded, after preparative TLC with hexane–AcOEt 2:1, a small amount of a substance with the same UV spectrum as *norketoyobirine*[8]: 344, 363, 380 sh mμ.

rac.-*Dihydrogambirtannine* (IX). Compound I (250 mg) were hydrogenated with PtO_2 (200 mg) in 30 ml AcOEt. The product was purified by chromatography through silica gel with hexane–AcOEt. The fractions with feeble yellow-green fluorescence were collected, to give 150 mg IX, m.p. 175° ($CHCl_3$–hexane).

LAH reduction of III

(a) Compound III (25 mg) suspended in dry ether were treated with 20 mg LAH and refluxed 30 min. The mixture was decomposed with some drops of water. The ether layer, dried with Na_2SO_4,

[33] G. Barger and C. Scholz, *Helv. Chim. Acta* **16**, 1343 (1933).
[34] R. B. Woodward and B. Witkop, *J. Am. Chem. Soc.* **70**, 2409 (1948).

gave on evapn. X, dec. > 250° ($CHCl_3$–ether). UV: 347, 354, 383 mμ (ε = 30,000, 30,400, 23,300). MS: *m/e* 316 (M^+, 100%), 315 (50%), 301 (26%), 297 (23%), 285 (17%), 284 (17%), 269 (13%), 256 (15%), 254 (15%), 128 (21%), 115 (15%).

(b) Compound III (200 mg) suspended in 250 ml dry ether were treated with 0·4 g LAH under nitrogen atmosphere, and refluxed. When TLC showed that there was no more III, and the amount of X initially formed had almost disappeared (3·5 hr), the mixture was decomposed with AcOEt, then with water. The product after evapn. of the ether layer was mixed with silica gel and chromatographed. The green fluorescent fractions, eluted with hexane–AcOEt 2:1, contained XI, which, crystd. from $CHCl_3$–hexane as orange crystals, melted at 200–205° dec, UV: 231, 256 sh, 306, 394 mμ (ε = 25,400, 13,500, 10,200, 31,700). MS: *m/e* 302 (M^+, 70%), 301 (100%). Found: 302·1404 ± 0·0015, calcd. 302·1419; composition $C_{20}H_{18}N_2O$. NMR (DMSO-d_6): 4·74, s, CH_2OH; 4·18, s, CH_2-21, 6·35, s, H-14; 11·20, s, NH; 3·10, center of ABCD: $CH_2(5)$–$CH_2(6)$; 6·9–7·7 (7 arom. protons).

The last fractions of the chromatography, with a pink fluorescence, eluted with AcOEt–MeOH 98:2, gave a red product, XVI, UV: 227·5, 249, 298, 382 sh, 402 sh, 475 mμ (ε = 11,100, 9500, 4170, 5370, 5200, 4400). NMR (DMSO-d_6): 4·95, s, CH_2OH; 4·83, t, (6·5 c/s), $-CH_2N^+$; 3·28, t (6·5 c/s), $CH_2(6)$; 8·45, s, H-21; 6·85–8·10, aromatic protons; NH, broad, hardly visible above the noise. MS: *m/e* 316 (M^+, 100%), 299 (40%), 287 (45%), 271 (15%), 158 (M^{2+}, 17%), 143 (double charged ion, 21%). Elemental composition of the important peaks:

316·1218 ± 0·0016	316·1212	$C_{20}H_{16}N_2O_2$	
299·1169 ± 0·0015	299·1184	$C_{20}H_{15}N_2O$	
287·1176 ± 0·0014	287·1184	$C_{19}H_{15}N_2O$	
271·1224 ± 0·0027	271·1235	$C_{19}H_{15}N_2$	ca. 30%
271·0865 ± 0·0014	271·0871	$C_{18}H_{11}N_2O$	ca. 70%

Carbinol XII. Compound XI (5 mg) in MeOH (10 ml) were hydrogenated in the presence of 10 mg PtO_2. Working up and crystn. from $CHCl_3$–hexane gave soft colorless crystals of racemic XII[22], m.p. 260°. UV: 224, 282, 290 mμ (ε = 48,400, 7380, 6150). MS: *m/e* 304 (M^+, 100%), 303 (93%), 285 (17%), 169 (52%), 144 (30%), 134 (17%). Elemental composition of the important peaks:

Found	Calc.	Composition
304·1570 ± 0·0015	304·1576	$C_{20}H_{20}N_2O$
303·1491 ± 0·0015	303·1497	$C_{20}H_{19}N_2O$
169·0762 ± 0·0008	169·0766	$C_{11}H_9N$
134·0729 ± 0·0007	134·0732	$C_9H_{10}N$

Levorotatory (3α) XII, $[\alpha]_D^{20} = -234°$ (95% EtOH, *c* 0·109) was prepared by reduction of 100 mg II with 100 mg LAH in 20 ml ether, refluxing 2 hr, working up as usual and chromatography through silica gel with hexane–AcOEt 1:2, yield 30 mg.

21-*Acetonyl-neooxygambirtannine* (XVIII). The preparation of 21-acetonylgambirtannine (XVII) by chromatography of the crude extract with hexane–acetone has been reported above. If the fractions containing XVII were exposed some days to the air, red products were formed. Rechromatography of the mixture through silica gel gave, besides mostly unreacted XVII, a red product which could be eluted only with AcOEt–MeOH 98:2. UV: 250 sh, 303, 390 sh, 410 sh, 505 mμ (ε = 21,600, 8900, 12,000, 20,000, 7450).

Reductions with $NaBH_4$

(a) To 8 mg IV dissolved in 10 ml MeOH excess $NaBH_4$ was added, until the solution was completely colorless. The soln was evapd, the residue taken up with water, centrifuged and washed with water. The product, m.p. 225°, appeared (MS) to be a mixture of XIV (M^+ = 348),

XV (main component, $M^+ = 316$), and I ($M^+ = 330$). TLC showed only a mixture of XIV and XV. I could then be an artifact by dehydration in the mass spectrometer.

(b) Compound XVI (8 ml) treated in the same way gave a product which appeared to be the carbinol XII by comparison of TLC behaviour, UV and mass spectra.

Acknowledgements—We thank Prof. C. Schöpf, Darmstadt, for a generous gift of neooxyberberine, Dr. A. L. Segre, Milano, for 100 Mc NMR spectra, Dr. G. Gallo, Lepetit Spa, Milano, for the ORD measurement, and Dr. F. Gambarin for kind information on the work of the late Prof. T. Pavolini. Last, but not least, we thank F. Hoffmann-La Roche and Co. AG, Basel, for the permission to use their mass spectrometer.