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STUDIES ON PLANTS—IX[1,2]

ISOLATION AND STRUCTURE OF RIBALINIUM SALTS

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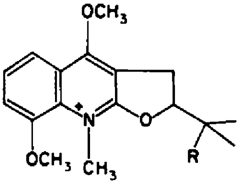
Abstract—From the trunk bark of *Balfourodendron riedelianum* (Engler) Engler (Rutaceae) the chloride and the perchlorate of the new quaternary alkaloid ribalinium (I) have been isolated.

According to functional groups analyses and UV absorption, I contains a 2,4-dialkoxy-1-methylquinolinium moiety (A), and the presence of a linearly fused oxygenated ring is indicated by the 4-quinolone nature of the nor-base (V) obtained by dequaternization of O-methylribalinium (IV). Coupled mass and NMR spectrometry established the oxygenated ring as 2-substituted-dihydrofurane; the side chain, an α-hydroxylated isopropyl fragment was proved by combined chemical, IR and NMR data. Finally, location of the phenolic function at position-6 by NMR measurements of I and V have led to the complete structure of the ribalinium cation.

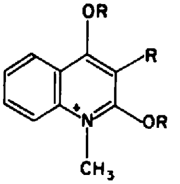
THE structure of a new quaternary alkaloid, named ribalinium (I), isolated from the trunk bark of *Balfourodendron riedelianum* (Engler) Engler (Rutaceae) has been elucidated.

This plant material contains a number of tertiary alkaloids:[1,3] in addition to five furoquinoline bases, the alkaloids evoxanthine, 1-methyl-2-phenyl-4-quinolone, balfourodine and isobalfourodine were found and a new quaternary alkaloid, O^4-methylbalfourodinium (II) salt,[3] closely related to the latter two bases, isolated.

Some of the alkaloids and related compounds are also present in other plants[4] belonging to the Rutaceae. In this connection, species of the *Lunasia* genus[5] contain enantiomorphs of some *Balfourodendron* alkaloids and a quaternary alkaloid,



II ; R = OH
III ; R = H



(A)

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[1] Part VIII: O. O. Orazi and R. A. Corral, *Anales asoc. quím. Argentina* **51**, 174 (1963).

[2] Presented in part at the 8th Latin American Congress of Chemistry (Buenos Aires, Argentina, September 1962).

[3] H. Rapoport and K. G. Holden, *J. Amer. Chem. Soc.* **81**, 3738 (1959); **82**, 4395 (1960).

[4] J. R. Price, *Fortschritte Chem. Organ. Naturstoffe* (Edited by L. Zechmeister) Vol 13; p. 302, Springer-Verlag, Wien (1956).

[5] As Ref guide, A. Rüegger and D. Stauffacher, *Helv. chim. Acta* **46**, 2329 (1963).

lunasine or methyllunacrinium[6] (III), structurally related to II, has also been isolated.

The ribalinium (I) salts were separated in about 0·3% yield (based on dry trunk-bark) either by partition chromatography of the total quaternary chlorides or through fractional crystallization of the corresponding perchlorates.

Rapoport and Holden[3] working with bark from young trees of Brazil found the content of II very high, but the bark samples[1] of old trees from our country yielded predominantly the quaternary compound (I).

Analytical data for the chloride, perchlorate and picrate of ribalinium (I) agree with the empirical formula $C_{18}H_{20}NO_4$ or $C_{18}H_{22}NO_4$ for the cation. Decision in favour of the former was reached by electronic integration of areas of the NMR spectrum of ribalinium chloride (60 Mc, deuterium oxide as solvent) which indicate 18 hydrogen atoms; two more must be added for the active hydrogens found by analysis, the signals of which are merged in the H–O–D peak of the solvent. Mol. wt determination by mass spectrometry of the transformation product, the nor-base (V), confirms this empirical formula and establishes that it also represents the molecular formula of I.

The quaternary nature of ribalinium (I) together with the IR spectrum, which shows a band at 3326 cm^{-1} and the absence of a carbonyl group, indicates that two oxygen atoms (according to the number of active hydrogens) are present as hydroxyl groups and the remaining two as ether linkages. The N-methyl group analysis and a comparison of the UV spectrum with those reported for methyllunacrinium (III) iodide[7] and O^4-methylbalfourodinium (II) salts,[3] indicate the presence of the structural moiety (A).

The hydroxyl groups of ribalinium (I) are represented as alcoholic and phenolic functions since the NMR spectrum indicates the presence of only three aromatic hydrogens and the compound gives a positive phenol reaction with aqueous ferric chloride; furthermore, methylation provides an O-methylderivative (IV) which shows (as iodide) a hydroxyl band at 3396 cm^{-1} in the IR but (as chloride) gives a negative ferric chloride reaction. The chloride of I is unaffected under hydrogenation conditions (Pd/C).

The molecular formula of the cation (I) in addition to the partial structure (A) requires an oxygenated ring either angularly or linearly fused.

This point was clarified by examination of the nor-base (V) prepared in accordance with a new dequaternization procedure[8] for this class of alkaloids. Nucleophilic abstraction of the methyl cation[7] from the methoxyl group was achieved by heating the iodide of IV in anhydrous pyridine. Since methyl iodide reconverts the nor-base (V) into the iodide of IV, no skeletal changes occur during the dequaternization process.

The nature of V is supported by elemental and functional group analyses, proton counting by NMR (in Cl_3CD at 100 Mc) and mass spectrometric determination of the mol. wt.; this base is a quinolone (adds one mole of Grignard reagent) and has its carbonyl group at position-4 as distinct from the 2-isomer since the IR spectrum[3,9]

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[6] J. R. Price, *Austral. J. Chem.* **12**, 458 (1959).

[7] S. Goodwin and E. C. Horning, *J. Amer. Chem. Soc.* **81**, 1908 (1959).

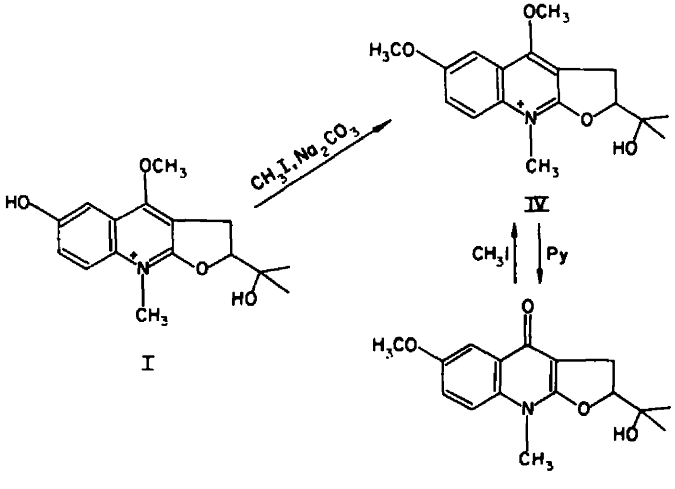
[8] Other methods[3,7] when applied to similar compounds furnished the tertiary bases in much lower yields; treatment of IV with lithium bromide in acetonitrile gave poor yields of V.

[9] A. V. Robertson, *Austral. J. Chem.* **16**, 451 (1963).

of V exhibits a maximum at 1552 cm^{-1} and, more important, the UV absorption suffers a pronounced change upon addition of acid,[3,10] and shows a minimum[11] at 265 mμ.

This result implies that the methoxyl group in the partial formula (A) is at position-4 and consequently the ring system is linearly fused.

In two related compounds, lunacrine and lunine, the oxygenated ring was proved by means NMR spectra to be a dihydrofurane on the basis of the presence of an unsubstituted isopropyl group;[12] in other instances (hydroxylunacrine and hydroxylunine[10]) the assignment of a dihydrofurane ring was based on the co-occurrence with the parent bases. In the case of other alkaloids[3,13] (v.gr. II and balfouridine) a comparison of UV absorption with appropriate models was used; but since the absorption data for dihydro-furanic and -pyranic rings are so close, it was decided that mass spectrometry[14] would provide a more reliable means of determining the size and substitution pattern of the oxygenated ring present in this alkaloid group.[15]



I

IV

V

The mass spectrum[16] of V shows at *m/e* 274 (M-15, from loss of methyl radical) a negligible intensity peak thus ruling out the six-membered ring present in some related alkaloids.[3,13] On the other hand (Fig. 1) one of the more prominent peaks at *m/e* 230 (M-59), with an intensity ratio of 300:1 in respect of the first mentioned, comes

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[10] S. Goodwin, A. F. Smith, A. A. Velásquez and E. C. Horning, *J. Amer. Chem. Soc.* **81**, 6209 (1959).

[11] M. F. Grundon and N. J. MacCorkindale, *J. Chem. Soc.* 2177 (1957).

[12] S. Goodwin, J. N. Shoolery and L. F. Johnson, *J. Amer. Chem. Soc.* **81**, 3065 (1959).

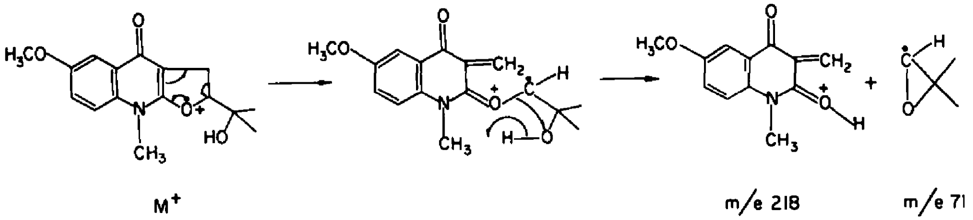
[13] H. C. Beyerman and R. W. Rooda, *Proc. Koninkl. Nederl. Akad. Wetensch.* **B-62**, 187 (1959); **B-63**, 154 (1960).

[14] K. Biemann, *Mass Spectrometry* p. 97. McGraw-Hill, New York (1962).

[15] When this study was in progress, application of mass spectrometry to a similar alkaloid appeared in the literature: F. Werny and P. J. Scheuer, *Tetrahedron* **19**, 1293 (1963).

[16] Mass spectrum run by direct inlet procedure: J. F. Lynch, J. M. Wilson, H. Budzikiewicz and C. Djerassi, *Experientia* **19**, 211 (1963).

from loss of C_3H_7O[17] and is indicative of a dihydrofuran ring in the base (V) which must also be present in the parent compounds (I and IV). The formation of the two strongest peaks (*m/e* 217 and 218) would be in accordance with position 2 rather than 3 for the attachment of the side chain, the nature of which is proved below.



The ion *m/e* 218 is strongly resonance-stabilized as it is the species *m/e* 217 arising from elimination of the hydrogen atom bonded to the oxygen.

The correctness of the inference regarding the attachment position for the side chain is fully corroborated by the NMR spectrum (Fig. 2). Signals of protons at C-2 and C-3 of dihydrofurane derivatives[12,18] can be ascribed to the multiplets at $\delta 4{\cdot}84$ and $\delta 3{\cdot}26$ respectively; this is further supported by double-resonance experiments (ω_1–ω_2 and the reverse, 156 and 157 c/s resp.) which prove that the involved hydrogens are mutually coupled.

The IR absorption and the active hydrogen analyses of the nor-base (V) and its progenitors (I and IV) together with the composition (C_3H_7O) of the side chain indicate that the latter contains an alcoholic function. Oxidation and acetylation attempts of IV under standard conditions for attacking primary and secondary alcohols, led to recovery of the starting material, thus indicating that the alcoholic group is tertiary. Accordingly, the chloride of I possesses a *gem*-dimethyl group on a C-atom carrying no hydrogen, which is in agreement with the Kuhn–Roth analysis (one C—CH_3) associated with the NMR data (in D_2O two unsplitted C—CH_3 signals, 8·0 c/s apart at 60 Mc and 5·1 c/s at 40 Mc). The nor-base (V) also shows two singlets (Fig. 2) corresponding to two shifted C-methyl groups (5·5 and 9·2 c/s apart at 60 and 100 Mc resp.).

The NMR absorption in the aromatic region (Fig. 3) of ribalinium (I) chloride represents an ABC[19] system (J_{AB} 9, J_{BC} 3, $J_{AC} \sim 0$ c/s) with a pattern corresponding to a 1,2,4-trisubstituted benzene; hence, the phenolic hydroxyl must be located either at position 6 or 7 in partial formulation (A). The NMR of the nor-base (V), being a 4-quinolone, must show the signal[9,12] of H_5 at significantly lower field than that of the other aromatic hydrogens.

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[17] Other fragments of 59 mass units [F. W. McLafferty, *Mass spectra correlations* p. 27 Amer. Chem. Soc. Washington, D.C. (1963)] can be excluded on the basis of the information already presented for compound V: molecular formula, functional groups analyses, IR spectrum (hydroxyl but no ester absorption) and the structural relationship with I and IV that defines the functional nature of the four oxygen atoms.

[18] L. M. Jackman, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* p. 87. Pergamon Press (1959).

[19] Values of δ (ppm downfield from $SiMe_4$) and J (c/s) given for the three-spin systems refer (apparent values) to the centre-gravity position and the observed separation of the pertinent peaks, resp.

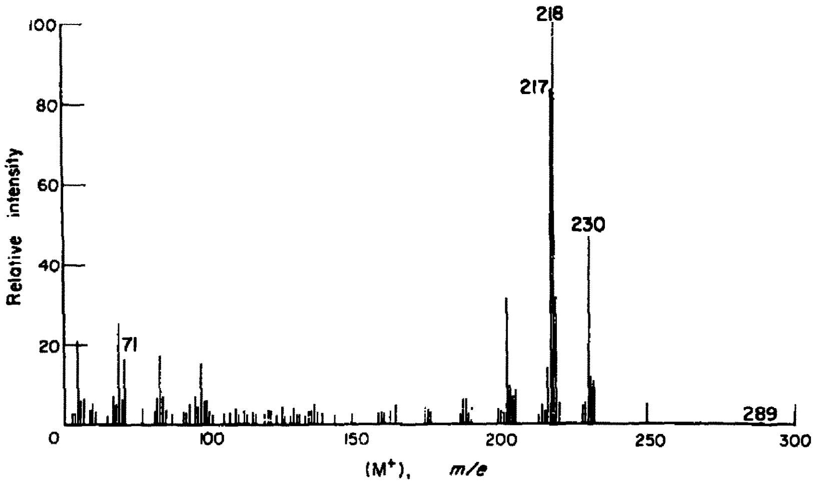

FIG. 1. Mass spectrum of the nor-base (V); except for M^+, peaks of relative intensity less than 2% are not plotted.

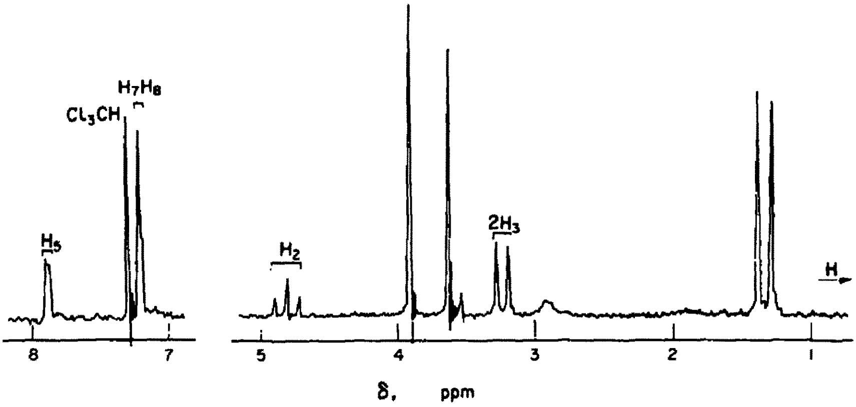

FIG. 2. NMR spectrum of the nor-base (V) in Cl_3CD (c, 1%), at 100 Mc and internal $SiMe_4$; the left region is an enlarged record.

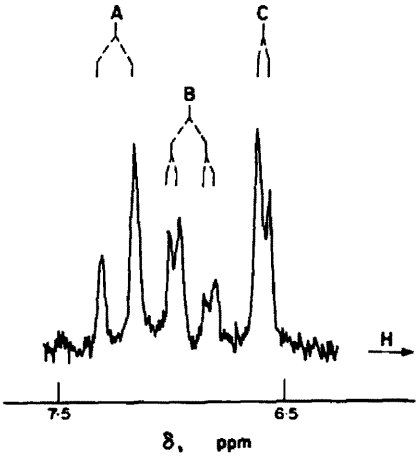

FIG. 3. NMR absorption in the aromatic region of ribalinium (I) chloride in D_2O (c, 10%), at 60 Mc and external $SiMe_4$.

In fact, V displays (Fig. 2) a signal of one hydrogen centred at 7·85 ppm and another at 7·18 ppm due to the remaining two (electronic area integration). This may be considered as a "deceptively simple" ABX system[20,21] ($\delta_A = \delta_B$; J_{AX} 2, $J_{BX} \sim$ 0 c/s). Both signals are divided by reciprocal coupling as proved by the spin-decoupling technique; by this means ($\omega_1 - \omega_2$ and the reverse, 63 c/s) both signals are converted into sharp singlets. Since J_{AX} is 2 c/s, H_5 has no hydrogen in *ortho*-position but it is coupled with a *meta*-hydrogen; therefore, the substituent must be placed at position 6 and the complete structure for the ribalinium cation is given by formula I.

EXPERIMENTAL

The m.ps (uncorrected) were taken in sealed capillaries. Unless otherwise stated the samples for analyses were dried at 56°/10^{-3} Torr; the microanalyses were performed by Dr. A. Bernhardt (Mülheim, Germany). The UV spectra in neutral or acidic medium were measured using alcohol 50° as solvent. In order to avoid an increase of pH observed during some ionic exchange experiments, the resin Dowex-2 chloride was stirred for 2 hr with 1N HCl, filtered and washed with water to remove the acid (Congo Red) and partially dried *in vacuo* at 40° (moisture content ca. 40%).

Isolation and characterization of ribalinium (I) *salts*

As it was detailed earlier,[1] the quaternary alkaloid fraction was precipitated as the reineckate (22 g from 2 K of dry trunk-bark) after complete extraction[22] of the tertiary bases at pH 11. The reineckate mixture was dissolved in 1·2 l. of acetone–MeOH (1:1) and after ionic exchange through a column of 250 g of Dowex-2 chloride furnished 12 g of a greenish yellow mixture of crude chlorides.

(a) *Isolation by partition chromatography.* Paper chromatography of this material using methyl ethyl ketone saturated with water plus the addition of 5% MeOH[23] proved to be the best system for the separation of the alkaloids.

The crude chlorides (6 g) were fractionated through a column of cellulose powder (450 g; Whatman, standard grade); small portions of the cellulose suspension in the above solvent system were transferred to the column and compressed by applying slight pressure with N_2. After collecting 190 fractions (20 ml each) with the same solvent mixture, the column was washed with MeOH.

According to the weight distribution, the first 190 fractions were made up as 50 fractions and examined by paper chromatography with the solvent system cited. The material corresponding to fractions 105 to 170 contained practically only one alkaloid (R_F 0·31). Recrystallization of this material from methanol-ethyl acetate provided 3 g of ribalinium chloride, m.p. 188–190° (gas evolution). The analytical sample was recrystallized from methanol; $[\alpha]_D^{24°}$ +40° (*c*, 1·25 in MeOH); λ_{max} 221 mμ (log ε 4·42), 247 (4·35), 293 infl. (3·83), 299 (3·88), 334 (3·65), λ_{min} 235 (4·28), 264 (3·40), 314 (3·41); ν_{max} (Nujol) *inter alia*, 3324, 1636, 1596, 1544, 1232, 976 cm^{-1}. (Found: C, 58·51; H, 6·30; N, 4·20; O, 19·38; Cl$^-$, 10·68; OCH_3, 9·45; N—CH_3, 4·61; C—CH_3, 3·89; active H, 0·60. $C_{16}H_{20}NO_4Cl$ requires: C, 58·98; H, 6·19; N, 4·30; O, 19·64; Cl, 10·89; one OCH_3, 9·52; one N—CH_3, 4·61; one C—CH_3, 4·61; two active H, 0·62%).

By mixing hot aqueous solutions of ribalinium (I) chloride and picric acid, the *picrate of* I crystallized almost quantitatively. The analytical sample was recrystallized from acetone and absolute alcohol, m.p. 192–193° (darkening, gas). (Found: C, 51·14; H, 4·46; N, 10·89; O, 33·59. $C_{16}H_{21}NO_4 \cdot C_6H_2N_3O_7$ requires: C, 50·97; H, 4·28; N, 10·80; O, 33·95%).

(b) *Isolation through the perchlorate.* The crude mixture of chlorides (1 g) was dissolved in 25 ml water and mixed with a hot solution of magnesium perchlorate (1·2 g) in 20 ml water. The resulting precipitate was crystallized from absolute ethanol to afford 0·59 g of ribalinium perchlorate, m.p.

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[20] R. J. Abraham and H. J. Bernstein, *Canad. J. Chem.* **39**, 216 (1961).

[21] As could be expected[20], changing the relative values of δ using dimethyl sulfoxide as solvent (at 60 Mc) a richer line pattern is obtained, constituting an ABC system with eight well resolved lines (δ_A 7·67, δ_B 7·60, δ_C 7·29 ppm; $J_{AB} \sim 0$, J_{AC} 2·9, J_{BC} 9·1 c/s).

[22] As far as ribalinium is concerned, the same results were obtained in a control experiment in which the removal of tertiary bases was made before an alkaline reaction (at pH 6·5) was reached.

[23] Cfr. with H. Schmid, J. Kebrle and P. Karrer, *Helv. Chim. Acta* **35**, 1864 (1952).

218–220° (darkening, gas). (Found: C, 49·40; H, 5·31. $C_{16}H_{20}NO_4.ClO_4$ requires: C, 49·30; H, 5·17%).

This product was identified by conversion into the corresponding chloride. An acetone–MeOH solution of the perchlorate (0·205 g) was poured through a column of resin Dowex-2 chloride (5 g); evaporation of the resulting solutions and crystallization of the residue from MeOH–ethyl acetate yielded 0·153 g of colourless crystals, m.p. 187–189° (gas); $[\alpha]_D$ +40° (*c*, 0·96 in MeOH) identical with ribalinium chloride described in (*a*) as demonstrated by coincidence of the IR spectra (KBr).

O^6-*Methylribalinium* (IV) *salts*

To a solution of ribalinium chloride (0·001 mole) in MeOH (12 ml) powdered anhydrous Na_2CO_3 (0·002 mole) was added and the mixture magnetically stirred for 15 min. After addition of excess MeI (2·5 ml), the mixture was heated at 45° with stirring in a stoppered flask during 24 hr. The filtered solution was acidified with acetic acid. After distillation of the solvent under red. press. the residue was crystallized from water (containing few mg Na_2SO_3) providing 0·345 g (80%) of *iodide* of IV, m.p. 188–190° (gas).

Some samples contained traces of I as indicated by paper electrophoresis (buffer pH 8; μ 0·02; 10 volt/cm; 8 hr) and detection of the spots with UV light. While IV gives an intense blue fluorescence, ribalinium (I) exhibits lower mobility and a very intense yellow spot.

The analytical sample was prepared by recrystallizations from alcohol, and dried to const. wt. at 100°/high vac.; m.p. 193–194° (gas); $[\alpha]_D^{18°}$ +20° (*c*, 1·18 in methanol); λ_{max} 222 mμ (log ε 4·67), 246 (4·48), 298 (3·96), 330 (3·76), λ_{min} 239 (4·44), 263 (3·48), 312 (3·56); ν_{max} (Nujol) at 3396 cm^{-1}. (Found: C, 47·00; H, 5·31; N, 3·10; O, 14·98; I^-, 29·60; OCH_3, 13·86; N—CH_3, 3·58; active H, 0·26. $C_{17}H_{22}NO_4I$ requires: C, 47·34; H, 5·14; N, 3·25; O, 14·84; I, 29·43; two OCH_3, 14·39; one N—CH_3, 3·48; one active H, 0·23%).

Treatment of the chloride of I with an equiv. of alkali and excess dimethyl sulphate in MeOH resulted the same O^6-methyl derivative (IV), which was isolated as the picrate (described below) and identified by its IR spectrum.

An attempt to methylate the chloride of I using excess ethereal diazomethane (from Diazald) distilled into a methanolic solution of I resulted in a mixture of a small quantity of chloride of IV together with unchanged starting material (according to paper electrophoresis).

By mixing hot aqueous solutions of the iodide of IV and excess magnesium perchlorate, an almost quantitative yield of the *perchlorate* of IV, m.p. 200–202° (darkening, gas), was obtained. For analysis it was recrystallized from water and dried to const. wt. at 80°/high vac. (Found: C, 50·10; H, 5·70; N, 3·66; O, 31·56; Cl, 9·13. $C_{17}H_{22}NO_4.ClO_4$ requires: C, 50·56; H, 5·49; N, 3·47; O, 31·70; Cl, 8·78%).

Picrate of IV. This was similarly prepared from the iodide and sodium picrate, m.p. 151–152° (from alcohol). (Found: C, 49·96; H, 4·92; N, 9·99; O, 34·89. $C_{17}H_{22}NO_4.C_6H_2N_3O_7.H_2O$ requires: C, 50·18; H, 4·76; N, 10·18; O, 34·88%. Dried to const. wt. at 100°/high vac., Found: O, 32·91; required without H_2O: O, 33·05%).

Dequaternization of IV *to the nor-base* (V)

A solution of the iodide of IV (0·001 mole) in 8 ml anhydrous pyridine was maintained 12 hr at 80° and then evaporated to dryness *in vacuo*. The residue was partitioned between $CHCl_3$ (20 ml) and water (4 ml) and the aqueous phase further extracted twice with $CHCl_3$ (10 ml each).

The combined chloroformic extracts were dried and evaporated, and a solution of the residue in absolute EtOH–$CHCl_3$ (1:2) was filtered through a column of 2 g alumina (Woelm, neutral, activ. II). Evaporation of the filtrate yielded crude *nor-base* (V) (0·265 g; 92%), m.p. 215–220° which recrystallized from alcohol to const. m.p. 223–224°; $[\alpha]_D^{20°}$ +47° (*c*, 1·11 in MeOH); λ_{max} 234 mμ (log ε 4·55), 243 (4·51), 301 (4·07), 319 (3·98), 333 (3·90), λ_{min} 239 (4·49), 265 (3·23), 313 (3·93), 329 (3·88); in 0·3 or 1N HCl, λ_{max} 224 (4·56), 241 (4·60), 296 (4·06), 324 (3·79), 332 infl. (3·77), λ_{min} 233 (4·54), 260 (3·46), 312 (3·65); ν_{max} (Cl_3CH; in 1600 cm^{-1} region) 1629, 1590, 1552, 1510 and 1476 cm^{-1}; ν_{max} (Nujol) at 3290 cm^{-1}. (Found: C, 66·60; H, 6·73; N, 5·12; O, 22·16; OCH_3, 10·76; N—CH_3, 4·84; active H, 0·40, CH_3MgI consumed, 2 moles; mol. wt. 289.[16] $C_{16}H_{19}NO_4$ requires: C, 66·42; H, 6·62; N, 4·84; O, 22·12; one OCH_3, 10·73; one N—CH_3, 5·20; one active H, 0·35%; mol. wt. 289).

A solution of V (5 mg) in MeOH (0·1 ml) and excess MeI (0·2 ml) was kept in the dark for one week at room temp. Evaporation under red. press. gave a colourless residue which was examined by chromatography using buffered paper at pH 2·2 and butanol equilibrated with the same buffer, as mobile phase.[24] UV fluorescence and iodoplatinic reagent showed up only two spots at coincident positions with those of the starting material (V) (R_F 0·60) and the O^6-methylribalinium (IV) iodide (R_F 0·41), run simultaneously on the same paper.

Acetylation and oxidation attempts of O^6-*methylribalinium* (IV)

(a) By anion exchange (resin Dowex-2 chloride), the iodide of IV (15 mg) was converted into the corresponding chloride which was carefully dried and then dissolved in 0·4 ml pyridine and 0·2 ml acetic anhydride. The solution was allowed to stand 48 hr at room temp; dilution with hexane brought about the separation of an oil which was repeatedly washed with this solvent and dried to constant wt (76°/vacuum) in order to eliminate last traces of pyridine. Water solution of this material was mixed with a saturated aqueous solution of picric acid, yielding 10 mg of a solid; the IR spectrum of which exhibited no carbonyl ester band and was coincident with that of picrate of IV.

(b) Chloride of IV, prepared as in (a), was added to a water solution (1·6 ml) containing CrO_3 (3 mg) and H_2SO_4 (5 mg); after 24 hr at room temp the picrate (15 mg) obtained as above, did not absorb in the IR carbonyl region and the spectrum was coincident with that of picrate of IV.

Acknowledgements—This work was generously supported by the Consejo Nacional de Investigaciones Científicas y Técnicas. Mass spectra and most of the NMR measurements (HR-60 and HR-100 Varian instruments) were run at Stanford University (U.S.A.) through the kindness of Prof. Carl Djerassi.

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[24] L. R. Goldbaum and L. Kazyak, *Anal. Chem.* **28**, 2889 (1956).