The presence or absence of Hodgkin's diesease in the spleen is therefore important and the reason for staging laparotomy and splenectomy. The spleen is involved in a nodular fashion, the earliest focal lesions occurring in marginal zone or follicles. Because a staging laparotomy may contain only a solitary tumor of one to a few millimeters in diameter, Maurer emphasizes that the spleen must be bread-loafed to slices less than 5 mm thicknesses. Histological diagnosis of Hodgkin's disease in any location depends upon the demonstration of Reed-Sternberg cells in an appropriate cellular background. Maurer goes on to discuss the impairment of cell mediated immunity in Hodgkin's disease before turning to malignant histiocytosis, as the last of the neoplasias considered. This is a systemic neoplasia of histiocytes with splenomegaly as the leading sign. The malignant histiocytes difusely infiltrate red pulp, and may encroach upon white pulp. Nuclear pleomorphism and erythrophagocytosis characterize the disease. Massive spenomegaly may occur as the only sign. Where the histiocytic and the phagocytic capacities are not evident immunocytochemical reaction for muramidase and chymotrypsin may be useful in defining the histiocytic nature of tumor cells. Differential diagnosis must consider hairy cell leukemia and diffuse histiocytic non-Hodgkin's lymphoma as well as Hodgkin's disease.

Sinzinger and Firbas present a most comprehensive and thoughtful study of splenic artery disease and disclose profound arteriosclerotic changes that occur in this vessel, having analyzed about 1500 human splenic arteries over a period of 10 years. The arteriosclerotic regions include fat infiltration, fibrous plaques, and calcification. The authors consider of the intima-media index, studies of the internal elastic membrane, discussion of smooth muscle cells, of parietal thrombus formation, of aging changes, and of the regulation of hemostasis. The authors demonstrate that at very early ages the human splenic artery is subject to severe arterosclerotic alterations. The reasons this artery undergoes such profound alterations and the clinical implications are not yet known.

Please turn to the summarized papers and to that of Weiss, Powell and Schiffman for references.

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Short Communications

Novel rearrangement of purines

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Summary. 6-Trichloromethyl-9-methylpurine (**1**) rearranges to 6-dichloromethyl-9-methyl-8-oxopurine (**2**) in aqueous mild acidic solution. The rearrangement is rationalized in terms of a reaction involving protonation, covalent hydration, prototropic equilibrium and/or a hydride transfer. An alternative mechanism involving a 'positive' halogen compound and hypochlorous acid as an intermediary is also proposed. Compound **1** condenses with 4,5-diaminopyrimidine to give the purine-pyrimidine Schiff base pair **4**.

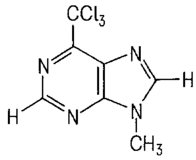
Key words. Purine rearrangement; synthetic purine-pyramidine pairs; 'positive' halogen compound; 6-trichloromethyl-9-methyl purine; 6-dichloromethyl-9-methyl-8-oxopurine; REDOX reaction.

'The essential feature is the base pairing'[2]. Since the elucidation of the structure of double-stranded DNA this phenomenon has continued to serve as a focus of activity both theoretically and experimentally[3]. Synthetic purine-pyrimidine pairs, particularly dinucleotide analogues of the type B-C$_n$-B', have been explored by Leonard as convenient models for studying interactions of nucleic acids bases in the absence of various complicating factors[4]. We have directed our efforts towards synthetic purine-pyrimidine pairs in which the bases are covalently connected by an amide or an imine linkage[5], as a lead to the development of anti-cancer agents[6].

Base pairs of the type Pu-CONH-Py were first prepared by Cohen and coworkers by condensations of 6-trichloromethylpurine with aminopyrimidines, and reportedly displayed unusual spectroscopic (UV and fluorescence) features[7-9]. In our attempts to synthesize Pu-CONH-Py pairs with fixed purine tautomers, we have investigated the behavior of N-methyl-6-trichloromethylpurines towards nucleophiles. We report a novel rearrangement of 6-trichloromethyl-9-methylpurine (**1**) into 6-dichloromethyl-9-methyl-8-oxopurine (**2**) in aqueous solution under mild acidic conditions. In the presence of aminopyrimidines, the reaction led to the formation of purine-pyrimidine Schiff base pairs.

6,9-Dimethylpurine[10-12] was chlorinated with sulfuryl chloride in trifluoroacetic acid at 60°C to give **1** as colorless crystals, m.p. 146–148°C in 57% yield[7]. ^{1}H NMR[13] δ (CDCl$_3$) 3.98 (s, N-CH$_3$), 8.24 (s, H-2), 9.06 (s, H-8). δ (Me$_2$SO-d$_6$) 4.03 (s, N-CH$_3$), 8.90 (s, H-2), 9.19 (s, H-8). ^{13}C NMR[13] δ(Me$_2$SO-d$_6$) 30.88 (q, J = 132.4 Hz, N-CH$_3$), 95.37 (s, CCl$_3$), 128.48 (s, C-5), 149.45 (d, J = 214.8 Hz, C-8), 150.95 (d, J = 192.2 Hz, C-2), 154.47 (s, C-6), 155.30 (s, C-4). δ(H-8) and δ(C-8) are shifted downfield by 0.6 and 2.6 ppm in **1** relative to 6,9-dimethylpurine[12] indicating a considerable localization of a positive charge at C-8. The NMR evidence does not indicate such an effect at C-2, the corresponding shifts of δ(H-2) and δ(C-2) being 0 and −1.4 ppm, respectively.

Heating an aqueous hydrochloric acid solution (5%) of **1** at 100°C under reflux for 24 h afforded **2** as colorless crystals, m.p. 189–191°C (dec) (C$_6$H$_6$ or Me$_2$CO) in quantitative yield. Mass spectrum m/e 234 (23%), 232 (36.5, M$^{+\cdot}$), 199 (50.3), 198 (15.4), 197 (100, M-Cl), 168 (5.7), 161 (6.7), 156 (18.2), 143 (5.9), 140 (6.6), 134 (31.9), 115 (13), 113 (37.7), 86 (20.9). ^{1}H



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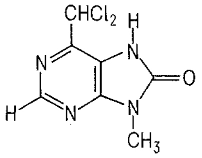

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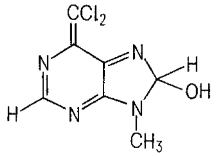

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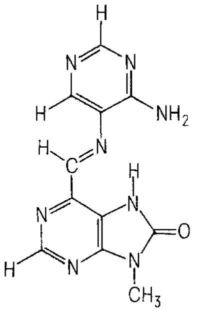

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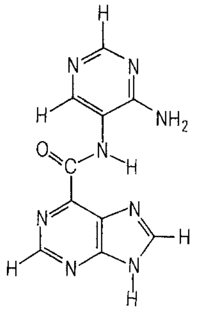

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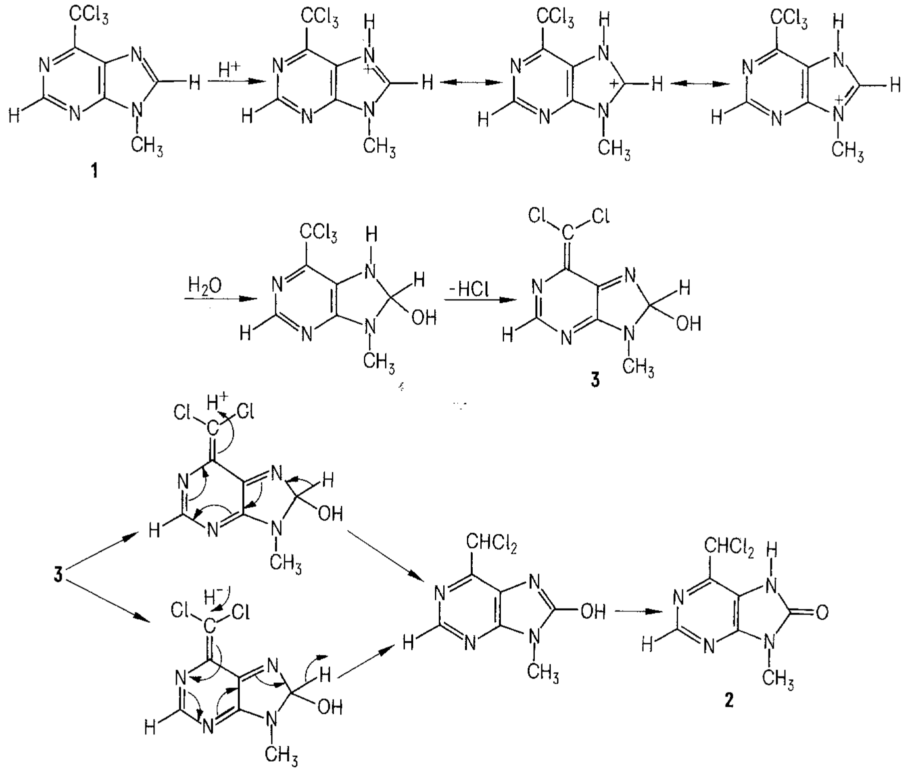
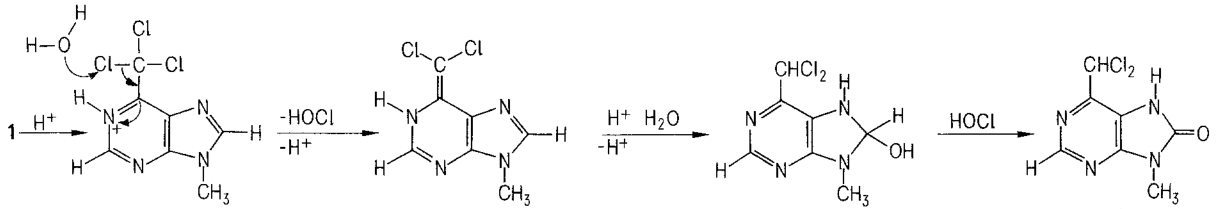
NMR[13] δ(Me_2SO-d_6) 3.43 (s, N-CH_3), 7.49 (s, $CHCl_2$), 8.77 (s, 1H, H-2), 11.87 (broad s, NH-7). ^{13}C NMR[13] δ(Me_2SO-d_6) 26.39 (q, J = 112.9 Hz, N-CH_3), 67.94 (d, J = 184.1 Hz, $CHCl_2$), 118.25 (s, C-5), 139.37 (s, C-6), 151.25 (d, J = 207.5 Hz, C-2), 153.46 (s, C-4), 154.30 (s, C-8). UV λ_{max}(EtOH) 281 nm (log ε 3.88). The structure of the $C_7H_6Cl_2N_4O$ species was elucidated by considering the changes in the ^{13}C NMR spectra upon rearrangement (Δδ = δ(**1**)–δk(**2**)). The CCl_3 singlet of **1** was shifted significantly upfield (Δδ = 27.3 ppm) and appeared in the rearranged product as a doublet characteristic of a $CHCl_2$ group, while the C-8 doublet of **1** was replaced by a singlet and shifted downfield (Δδ (C-8) = –5.1 ppm). (Δδ (C-5) = 10.2 ppm and Δδ (C-6) = 15.1 ppm should be noted). The missing clue was provided by the infrared spectrum of **2** which showed a strong carbonyl stretching mode at 1720 cm^{-1} and an NH mode at 3180 cm^{-1} characteristic of an 8-oxopurine. The reaction is acid catalyzed, and in the absence of a buffer, is self catalyzed. At pH = 3.00, the reaction is quite slow and a gradual rise in pH followed by a fall in pH is observed, as expected from the stoichiometry of the HCl elimination.



Scheme 1. Proposed mechanism for the **1**→**2** rearrangement (covalent hydration-HCl elimination route).



Scheme 2. Proposed mechanism for the **1**→**2** rearrangement ('positive halogen' route).

The rearrangement of **1** into **2** may be rationalized in terms of the following mechanistic sequences (scheme 1).

a) A protonation of **1** at N-7 to give a positively charged species in which the electrophilic character at C-8 is enhanced[14, 15].

b) A nucleophilic attack by H_2O at C-8 (covalent hydration of a purine[16]) followed by an $\alpha(N7)\delta$-hydro-chloro-elimination to give the key tautomeric intermediate (**3**).

c) The intermediate **3** which resembles the intermediates in the Cannizzaro reaction might undergo a rearrangement following two different paths: 1. A series of prototropic equilibria involving a proton transfer from the medium to the 6-dichloromethylene carbon and a concomitant proton elimination from C-8. 2. Alternatively, the strong electron-donating character of the oxygen and nitrogen substituents at C-8 of **3** greatly facilitates the ability of H-8 to leave with its electron pair, resulting in a hydride transfer of H-8 to the 6-dichloromethylene carbon[17] perhaps through stacking of two purine moieties. Indeed, hydride shift products of diamine-dialdehyde condensations in acetic acid or methanol have been reported[18].

d) A tautomeric equilibria of an 8-hydroxypurine to the corresponding 8-oxo-7H-purine derivative (**2**). The analogy with the Cannizzaro reaction may be extended further. Radical species have recently been shown to participate in the Cannizzaro reaction[19]. The possible involvement of radical intermediates in the present rearrangement cannot be ruled out at this point. However, the reaction could proceed under an argon atmosphere in degassed aqueous acidic solution in the dark.

An attractive plausible mechanism for the **1**→**2** rearrangement[1] involves hypochlorous acid as an intermediary (scheme 2)[20]. **1** is first protonated at N-1 and then H_2O, acting as a nucleophile, abstracts a 'positive' chlorine from the 6-trichloromethyl group to give HOCl and 6-dichloromethylene-9-methyl-1H-purine. Covalent hydration of the latter intermediate at C-8 (and N-7) followed by a prototropic shift afford the 6-dichloromethyl-8-hydroxy-9-methylpurine which is oxidized at C-8 by HOCl, leading to **2**. The **1**→**2** rearrangement may perhaps be considered a REDOX reaction, namely, 'a chemical reaction involving reduction and accompanying oxidation'[21]. It should be noted, however, that the mechanisms of the rearrangement outlined above (schemes 1+2) are only proposed mechanisms and none of them have been established.

A condensation of **1** with 4,5-diaminopyrimidine in aqueous hydrochloric acid solution (pH 3) at 78–80 °C for 48 h gave *E*-N-(9-methyl-8-oxo-6-purine-methylene)-4-amino-5-pyrimidine amine (**4**), m.p. > 300 °C. Mass spectrum m/e 270 (15.5%, $M^{+\cdot}$). IR ν_{max} (KBr) 3170 (broad), 2920, 1723, 1640, 1593, 1572, 1500, 1400, 1363, 1350, 1240, 1122, 980, 890 cm^{-1}. UV λ_{max} (0.1 N NaOH) 250 nm (log ε 4.62), 370 b (4.55). λ_{max} (0.1 N HCl) 265 nm (log ε, 4.55), 335 b (4.28). ^1H NMR $\delta(Me_2SO\text{-}d_6)$ 3.48 (s, N-CH_3), 7.46 (s, NH_2), 8.32 (s, H-6′(Py)), 8.43 (s, H-2′(Py)), 8.83 (s, HC = N), 8.85 (s, H-2), 12.36 (broad s, NH-7). δ(H-2′) and δ(H-6′) are shifted downfield by 0.50 and 0.65 ppm relative to 4,5-diaminopyrimidine, indicating a reduced delocalization of the nonbonding electrons of the azomethine nitrogen into the pyrimidine ring and a substantial twist of the pyrimidine ring vis-à-vis the coplanar PuCH = N-moiety. The lowfield absorption shift of N(7)-H at 12.36 ppm and the maximum at about 350 nm in the electronic absorption spectrum point towards a hydrogen bond between N(7)H and the azomethine nitrogen in the *E* configuration. **4** could also be obtained by a condensation between **2** and 4,5-diaminopyrimidine in boiling ethanol solution. The fact that 6-trichloromethylpurine did not undergo an analogous rearrangement under comparable conditions but condensed normally with aminopyrimidines to give purine-pyrimidine amide pairs e.g., N-(4-amino-5-pyrimidinyl)purine-6-carboxamide (**5**)[5, 7] is probably due to the presence of H-9. Under mild acidic conditions this facilitates the elimination of HCl without prior protonation, to give a neutral intermediate ($Pu = CCl_2$)[14] in which the electrophilic character at C-8 is diminished. A nucleophilic attack would then favor the 6-dichloromethylene carbon. However, at pH < 1, under conditions of permanent protonation, 6-trichloromethylpurine does indeed undergo a similar rearrangement, leading to 8-oxo-purine-6-carbaldehyde. Consistently, 6-trichloromethyl-7-methylpurine (prepared by chlorination (SO_2Cl_2/CF_3CO_2H) of 6,7-dimethylpurine[12]) did not undergo an analogous rearrangement. It is first protonated at N(1) (^1H NMR[13] $\delta(Me_2SO\text{-}d_6)$ 4.38 (s, N-CH_3), 9.08 (s, H-8), 9.15 (s, H-2), 10.58 (s, NH)), then at N(9) (^1H NMR[13] $\delta(Me_2SO\text{-}d_6)$ 4.59 (s, N-CH_3), 9.06 (s, H-8), 9.16 (s, H-2), 9.96 (s, NH)) and the protonation may be followed by a nucleophilic attack of H_2O (covalent hydration[16]) at C-8. However, resonance considerations indicate that the resulting species would not eliminate HCl, and thus render the rearrangement inaccessible. Alternatively, the lack of rearrangement of 6-trichloromethyl-7-methylpurine may well be due to a large steric hindrance opposing formation of the critical intermediate[1].

The rearrangement of **1** may serve as a chemical simulation to the enzymatic oxidation of purines at position 8 by xanthine oxidase. It has been suggested that the enzymatic oxidation is, overall, one of coupled transfer of a hydride from the xanthine to the complex electron sink of the enzyme (Enzyme–Mo=S acceptor) and of an electron pair to molybdenum, while the C-8 carbonium ion reacts with a nucleophile[22, 23]. Synthetically, the rearrangement provides a convenient entry to 8-oxopurine-6-carbaldehyde which is a promising synthon of C-6 nucleosides.

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[2] Judson, H.F., interview with F.H.C. Crick, in: The Eighth Day of Creation, p. 181. Simon and Schuster, New York 1979.

[3] Reviews: Löwdin, P.-O., Adv. Quantum Chem. *2* (1965) 213; Ladik, J.J., Adv. Quantum Chem. *7* (1973) 397; Ts'o, P.O.P., in: Basic Principles in Nucleic Acid Chemistry, p. 453, vol. 1, Ed. P.O.P. Ts'o. Academic Press, New York 1974.

[4] Browne, D.T., Eisinger, J., and Leonard, N.J., J. Am. chem. Soc. *90* (1968) 7302; Leonard, N.J., Accounts chem. Res. *12* (1979) 423, and references therein.

[5] Agranat, I., and Barak, A., Heterocycles *11* (1978) 213.

[6] Montgomery, J.A., J. med. Chem. *23* (1980) 1063.

[7] Cohen, S., in: The Purines – Theory and Experiment, The Jerusalem Symposia on Quantum Chemistry and Biochemistry IV, p. 539. Eds E.D. Bergmann and B. Pullman. The Israel Academy of Sciences and Humanities, Jerusalem 1972.

[8] Lachman, Ch., and Cohen, S., Israel J. Chem. *6* (1968) 131.

[9] Cohen, S., Gabor, G., Rubinstein, R., and Vincze, A., Chem. Phys. Lett. *42* (1976) 456.

[10] Vincze, A., and Cohen, S., Israel J. Chem. *4* (1966) 23.

[11] Lam, F.L., and Parham, J.C., Heterocycles *9* (1978) 287.

[12] Barak, A., and Agranat, I., Nouv. J. Chim. *5* (1981) 491.

[13] The ^1H and ^{13}C NMR spectra were obtained with a Bruker WH-300 Pulse-FT spectrometer operating at 300.133 MHz (^1H) and 75.46 MHz (^{13}C). The data are reported in ppm, downfield from Me_4Si (δ untis). Long range couplings were omitted.

[14] Cohen, S., and Dinar, N., J. Am. chem. Soc. *87* (1965) 3195.

[15] When the reaction was carried out in D_2O-DCl (5%), 6-dichlorodeuteromethyl-9-methyl-8-oxopurine was formed. A deuterium exchange at C-8 probably preceded the nucleophilic attack[17].

16 Albert, A., Angew. Chem., internat. Edn, Engl. *6* (1967) 919; Albert, A., Adv. heterocyclic Chem. *20* (1976) 117; Albert, A., J. chem. Soc. (B) *1966*, 438.

17 7,8-Dihydro-7,9-dimethylguanine anion may act as a donor of hydride ion from position 8: Hecht, S.M., Adams, B.L., and Kozarich, J.W., J. org. Chem. *41* (1976) 2303.

18 Bindra, A.P., and Elix, J.A., Tetrahedron *25* (1969) 3789.

19 Chung, S.-K., J. chem. Soc. chem. Commun. *1982*, 480.

20 For a recent review on 'positive halogen' compounds, see Foucaud, A., in: The Chemistry of Functional Groups, Supplement D, The Chemistry of Halides, Pseudo-halides and Azides, part 1, pp. 441–480. Eds S. Patai and Z. Rappoport. John Wiley and Sons, Chichester 1983.

21 Orchin, M., Kaplan, F., Macomber, R.S., Wilson, R.M., and Zimmer, H., The Vocabulary of Organic Chemistry, p. 317. Wiley-Interscience, New York 1980. Although there is no net change in the oxidation state of the carbons in the molecules of **1** and **2**, the oxidation state of C-8 is increased while that of 6-methyl carbon is decreased. Indeed, the latter mechanistic sequence (scheme 2) involves reduction of 6-trichloromethyl carbon by H_2O which is oxidized into HOCl, with eventual oxidation of C-8 by the intermediate HOCl.

22 Walsh, C., Enzymatic Reaction Mechanisms, p. 438. W.H. Freeman and Company, San Francisco 1979.

23 Bray, R.C., Gutteridge, S., Stotter, D.A., and Tanner, S.J., Biochem. J. *177* (1979) 357.

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Flavonoid profiles of certain species of *Rhynchosia* of the family Leguminosae (Fabaceae)

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Summary. A survey of seventy naturally occurring populations of *Rhynchosia* representing seven species of different subgenera revealed the occurrence of C-glycosides, O-glycosides, prenylated flavonoids and aglycones. *Rhynchosia albiflora (R. cyanosperma)* differs in gross morphological features and seed coat color and in its flavonoid composition from the other representative species of *Rhynchosia.*

Key words. Rhynchosia gen.; flavonoids, leaf.

The genus *Rhynchosia* is widely distributed with several species occurring in mountainous regions in the tropics. The wide range of morphological features selected to classify the genus has often resulted in anomalies in the systematic position of certain species, as is evident in the regional floras of Hooker[2], Cooke[3] and Duthie[4] and in the classification of Roxburgh[5] and Bentham and Hooker[6]. As described in the floras, the basic similarities of various species of *Rhynchosia* are, pinnate leaves, compressed pods and calyx not accrescent. The division of the genus *Rhynchosia* into various subgenera involves differences in enlargement of the fruiting calyx in size and breadth, and the presence or absence of the arillus on the seed.

Cylista tomentosa has been re-classified as a member of the genus *Rhynchosia.* It was originally called *R. cyanosperma* but is now called *R. albiflora.* We therefore considered it would be valuable to compare the flavonoid profiles of seven indigenous species of five subgenera *(Phyllomatia, Eurhynchosia, Cyanospermum, Ptychocentrum* and *Nomismia),* to see whether *Rhynchosia albiflora* would fit comfortably into the genus with regard to its flavonoid composition or whether it differs from the other representatives, as shown in morphological details. Though the members of Fabaceae have been widely surveyed for morphological and chemical characteristics, the details at the level of the species included in various genera of the family have not been adequately enough worked out to arrive at a logical phylogenetic arrangement of the species at generic level. Earlier studies have shown the occurrence of flavonoids in three different species of *Rhynchosia*[7]. The present survey encompasses three more species of *Rhynchosia (R. heynei, R. capitata* and *R. sericea)* and compares the results with those for *R. albiflora* (table).

Flavonoids which were reported in earlier studies and in three species in the present study are different from those of *R. albiflora.* A minimum of 9 populations of 7 species of *Rhynchosia* (80 g dry wt per population of each species) were examined for flavonoid constituents of the leaves. Isolation of flavonoids and their glycosides, hydrolytic procedures[8] and spectral analysis were carried out using standard methods[9]. Flavonoids were exhaustively extracted using both acetone and methanol, reduced to dryness and loaded on column, paper and thin-layer chromatograms[10]. They were then reisolated and characterized using normal analytical procedures (UV, IR, NMR and MS)[9]. Sixteen different flavonoid compounds have been identified from the selected species (table). Of these, there are eight C-glycosides, three O-glycosides, two prenylated flavonoids (dihydroflavonol and the corresponding flavonol), a flavone, a flavanone and a 5-deoxyflavonol. The C-glycosides vitexin (8-C-β-D-glucopyranosyl apigenin), isovitexin (6-C-β-D-glucopyranosyl apigenin), orientin (8-C-β-D-glucopyranosyl luteolin), isoorientin (6-C-β-D-glucopyranosyl luteolin) and vicenin-2 (6,8-di-C-β-D-glucopyranosyl apigenin) are invariably present in *R. rufescens, R. heynei, R. capitata, R. beddomei, R. minima*[11,12] and *R. sericea,* while lucenin-2 (6,8-di-C-β-D-glucopyranosyl luteolin) is common to *R. rufescens, R. beddomei* and *R. minima.* In the disputed species *Rhynchosia albiflora* none of the C-glycosides is present, instead, it was found to possess two O-glycosides namely rutin (quercetin-3-rutinoside) and kaempferol-3-rutinoside and two prenylated flavonoids namely tirumalin[13] (8-C-prenyltaxifolin 7,4′-dimethyl ether) and rhynchospermin[14] (8-C-prenylquercetin 7,4′-dimethyl ether), and is thus very different in its flavonoid composition. However, *R. beddomei* showed the occurrence of the maximum number of flavonoid constituents, which included apigenin, naringenin and rhynchosin (5-deoxyquercetagetin) and it is the only species which possessed O-glycosides close to those of *R. albiflora.* Rhynchosin[15], a compound devoid of 5-hydroxyl groups occurring in *R. beddomei,* is considered to be an isolated character[16].

It is now clear that *R. albiflora* (Sims.) Alston, *(R. cyanosperma)* which has undergone a name change and a generic transfer from *Cylista tomentosa* Roxb., to *Rhynochosia* is quite distinct from other species of *Rhynchosia* not only in its general morphology and blue seeds but also in its flavonoid compounds. The other species of *Rhynchosia* which were studied here shared many morphological features as well as