TABLE I

SPECIFIC ROTATION OF 0.80% GAD IN 0.02–0.10 *M* SODIUM SULFATE OR PHOSPHATE (+ NaOH), *T* 25°

λ, mμ	Native GAD, *p*H 6.9–8.6	*p*H 9.5, 30 minutes after adding NaOH[a]	*p*H 9.5, 4 hours later
546.1	+13° ± 5	−22° ±3	−49° ± 4
492		−28	−72
435.8	+21	−50	−97
404.7	+20	−63	−122
366	+9	−121	−164
b_0	−170 ± 40	−357	−70

[a] These values are corrected for the change occurring during observation.

Although the accuracy, because of the weak rotatory power and turbidity, was low, the dextrorotation was ascertained using three different preparations, *e.g.*, the $[\alpha]_{435.8}$ of one of them (in 0.02*M* sodium sulfate, *p*H 7.1) was +24°, while another preparation (in 0.1 *M* phosphate, *p*H 6.9) yielded at the same wave length +18°, and a third (in 0.01 *M* sulfate, *p*H 7.4) +19°. Treatment of the native enzyme with sodium decyl sulfate (0.05 *M*, *p*H 7.8) at 25° resulted in a negative shift of the specific rotation ($[\alpha]_{435}$ −87°), and upon this treatment the turbidity disappeared. The λ_c of the detergent treated GAD was 247 mμ.

The rotatory data of the native GAD did not fit the one-term Drude rule. The data then were evaluated according to the Moffitt–Yang method,[10] *i.e.*, the $[\alpha]_\lambda \times (\lambda^2 - \lambda_0^2)$ values were plotted against $\lambda^4/(\lambda^2 - \lambda_0^2)$, where λ_0 is 212 mμ, and the b_0 values of the Moffitt–Yang equation[10] were calculated (see Table I). The b_0 for the detergent treated GAD was found to be −98. (The refractive index and residual molecular weight factors were disregarded, since they do not affect b_0 greatly.[11]) Recently the b_0 of another preparation of native GAD was rechecked with an improved Rudolph model 80-AQ6 instrument, and the b_0 was found to be −208 (±20). The $[\alpha]_\lambda = f(\lambda)$ curve had a flat minimum at 290–300 mμ. The Moffitt–Yang equation was applicable only within the near ultraviolet and visible spectral range.

The enzymic activity was tested according to Strecker,[12] and the neutral solutions were found as active as the most active preparations described in the literature.[12,13,14] At *p*H 8.6, however, the activity was considerably diminished, whereas no significant change could be seen in the rotatory power. Denaturation with alkali at *p*H 9.5 (4.5 hours at 25°) or with sodium decyl sulfate resulted in complete inactivation.

Since the molecular weight of native GAD is known to be about 10^6, and since the enzyme dissociates and aggregates readily,[13] it seems likely that the $M = 10^6$ particle may be composed of several polypeptide chains or subunits.[15] Terminal amino acid analysis of three specimens after Sanger[16] showed that 17–23 moles of N-terminal alanine and 1–2 moles of N-terminal aspartic and/or glutamic acids are present per mole of enzyme. Thus the $M = 10^6$ particle is composed at least of 17–23 peptide chains. After adding the decyl sulfate, a complete dissociation occurred, since a low s_{20}^0 of 3.7 *S* and a low intrinsic viscosity of 0.025 dl./g. was observed. Application of the Scheraga–Mandelkern treatment[17] by taking a β factor of 2.16, yielded for the subunits an *M* of 43,000. This is in reasonable agreement with the N-terminal group analysis, *i.e.*, each subunit possibly represents one polypeptide chain.

The results indicate that GAD is a protein of unusual conformation. The negative b_0 and the dextrorotation of the native enzyme suggest the presence of α-helical conformation.

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THE COPPER SALT CATALYZED PEROXIDE REACTIONS

Sir:

The versatility of the copper salt catalyzed reaction of peroxides with various substrates has been well described.[1,2,3,4,5] In particular, the reaction between peroxides and olefins has been postulated to proceed via a complex containing copper, peroxide and olefin.[1,4,5] This termolecular mechanism is based in part on the *non-rearrangement* of allylic systems.[1,4]

We have examined this reaction between *tert*-butyl peracetate and perbenzoate with the three isomeric butenes in the presence of cuprous bromide at 75–85°. The stoichiometry is described by the equation

$$RCO_3Bu\text{-}t + C_4H_8 \rightarrow RCO_2C_4H_7 + t\text{-}BuOH$$

The over-all yields of butenyl acetates and benzoates are in the range of 70–85%. The compositions of the butenyl ester mixtures were examined by gas–liquid chromatography and found to be *invariant* (within 5%) with the reactant butene. Thus, butene-1, *cis*-butene-2 and *trans*-butene-2 all yield a mixture of butenyl esters consisting of 89–94% 3-acyloxybutene-1 and 11–6% crotyl esters. Under the conditions of these experiments there is no allylic isomerization of either the reactant butenes[6] or product esters.

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The isomer ratios of the butenyl esters are also insensitive to solvent composition, which ranged from benzene, acetonitrile, methanol, *tert*-butyl alcohol, 20% aqueous *tert*-butyl alcohol to acetic acid. In the last solvent *tert*-butyl perbenzoate and butene-2 yielded the same mixture of butenyl acetates[7] as was derived from *tert*-butyl peracetate in benzene. In the other protic solvents (alcohol and water) there were formed besides the butenyl esters, products (butenyl ethers and alcohols arising from the solvent. The distributions between the 3-but-1-enyl and crotyl derivatives in these latter products were the same as those of the corresponding esters.

These experiments indicate that the butenyl intermediate leading to products is *free* to undergo isomerization. We prefer to postulate the reaction occurring via the steps

$$RCO_3Bu\text{-}t + Cu(I) \xrightarrow{a} RCO_2Cu(II) + t\text{-}BuO\cdot$$

$$t\text{-}BuO\cdot + C_4H_8 \xrightarrow{b} t\text{-}BuOH + C_4H_7\cdot$$

$$C_4H_7\cdot + Cu(II)O_2CR \xrightarrow{c} C_4H_7O_2CR + Cu(I), \text{ etc.}$$

Ethyl radicals derived in 70–80% yield from the cuprous-catalyzed decomposition of *tert*-amyl perbenzoate in benzene, methanol or cumene yielded ethylene (90–98%) and ethane (7–1%).

$$t\text{-}C_5H_{11}O\cdot \rightarrow CH_3CH_2\cdot + CH_3COCH_3$$

$$CH_3CH_2\cdot + Cu(II)O_2R \xrightarrow{c'} CH_2{=}CH_2 + RCO_2H + Cu(I)$$

The catalysis by cuprous ion (step a) and the oxidation-reduction reactions[8] (steps c and c′) will be commented on later.

Similar results have been reported to us by D. B. Denney, who currently is investigating the allylbenzene–propenylbenzene system.

(7) The butenyl benzoates did not interchange with solvent to form butenyl acetates under these conditions.

(8) H. E. De La Mare, J. Kochi and F. F. Rust, *J. Am. Chem. Soc.*, **83**, 2013 (1961).

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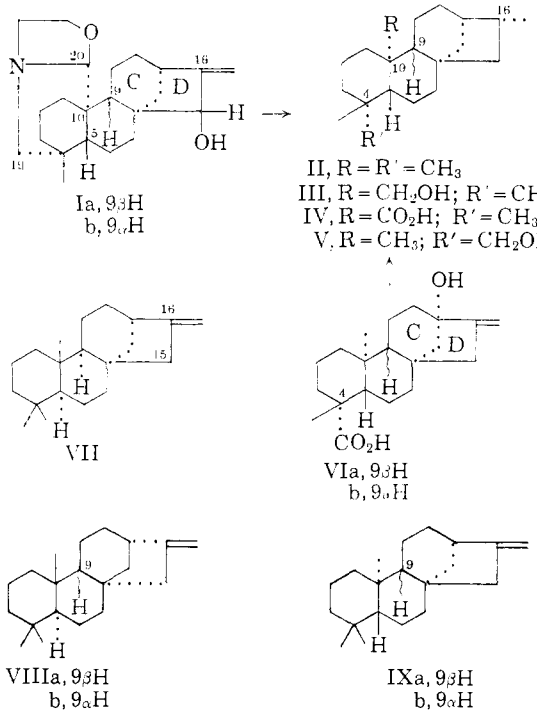
A DIRECT CORRELATION OF THE DITERPENE ALKALOIDS AND HYDROCARBONS OF THE PHYLLOCLADENE GROUP. INTERCONVERSION OF GARRYFOLINE AND STEVIOL[1]

Sir:

Recently,[2] we reported on the transformation of the alkaloid garryfoline (I)[3] into the hydrocarbon II, which was not identical with either of the two C-16 isomeric hydrogenation products[4] of phyllocladene (VII), a diterpene of known relative and absolute stereochemistry.[5] We have now found that our hydrocarbon II is identical (mixture melting point, gas-phase chromatographic mobility) with a newly synthesized[6] specimen of "Stevane-B" (m.p. 54–55°, $[\alpha]_D$ −56°) derived from steviol.[7] The precursor alcohol III[2] from garryfoline (I) proved to be completely different from the corresponding isomeric alcohol (m.p. 142–144°, $[\alpha]_D$ −62°), which now has been prepared from steviol (VI) and related to "Stevane-B" (II). It follows, therefore, that the alcohol from steviol must be V and that the carboxyl group in steviol (VI) must be located at C-4, thus settling the remaining structural question of this interesting diterpene acid. Further confirmation of this conclusion could be provided by pK^*_{MCS} measurements[8] (kindly performed by Dr. V. P. Arya) on five different steviol and isosteviol derivatives, which ranged between 8.52–8.68, in contrast to pK^*_{MCS} 9.49 reported earlier[2] for the isomer IV with the carboxylic acid at C-10. The apparent dissociation constants of steviol (VI) and its derivatives are in excellent agreement with that[8] of deoxypodocarpic acid[9] (pK^*_{MCS} 8.45), which also possesses an axial carboxyl group at C-4 and an A/B *trans* ring juncture.

"Stevane-A,"[6] the C-16 epimer of "Stevane-B" (II), has been shown[6] to be identical with (−)-α-dihydrokaurene, the principal hydrogenation product of (−)-kaurene.[10,11] "Stevane-B" (II), therefore, is (−)-β-dihydrokaurene (m.p. 51–52°)[10] and the interconversions shown thus represent the



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